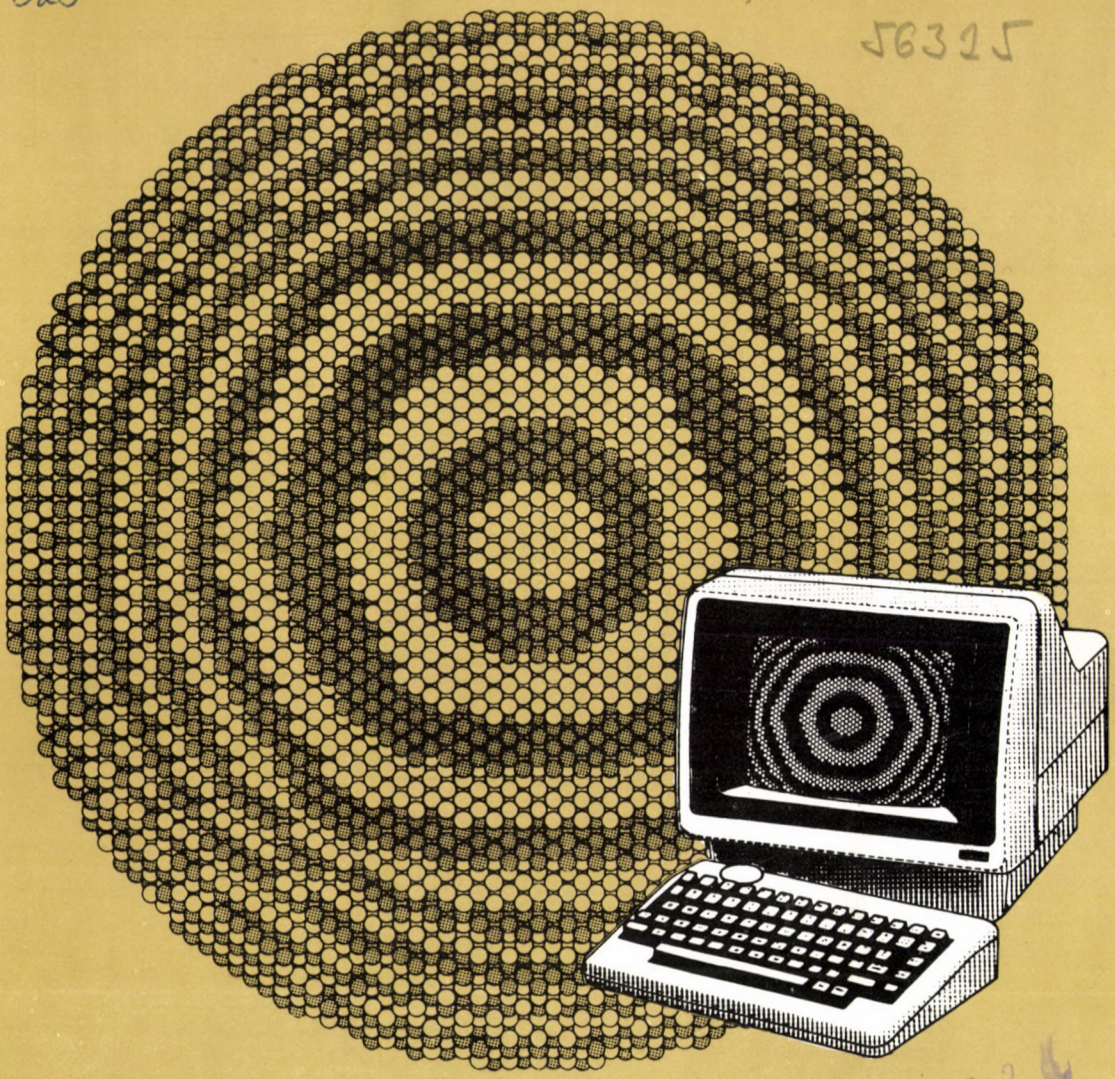


biotech-info

Special issue 1990. HU ISSN 0237–0115

OMIKK AGROINFORM

BIOTECH-INFO

Az OMIKK - AGROINFORM - OMFB közös kiadványa

Készül az **MTA Könyvtára** és a **Szegedi Biológiai Központ** munkatársainak közreműködésével

Kiadja az
Országos Műszaki Információs Központ és Könyvtár
Bp. VIII. Múzeum u. 17.
Postacím: Bp. Pf. 12. 1428

A szerkesztőség telefonszáma: **1-382-915**

A kiadásért felelős: Jánszky Lajos
Készült az OMIKK nyomdájában
1011 Bp, Gyorskocsi u. 5-7.
Felelős vezető: Tóth Károly

BIOTECH-INFO

Special Issue

BIOTECHNOLOGICAL PUBLICATIONS OF HUNGARIAN AUTHORS IN 1990

Edited by:
Mr László Kállai

Budapest, 1991

BIOTECH-INFO

Special Issue

1990

The publication contains abstracts and annotations of selected works published by Hungarian authors in the technical literature on biotechnology in Hungary

---

editor-in-chief:
Prof.Dr.László Kállai

editor:
Mrs Zsuzsanna Horváth-Fazekas

technical editor:
Márta Labancz

editorial office:
National Technical Information Centre and Library
Budapest, Hungary
H-1428 P.O.B.12
Telephone 138-2915
138-2300\192
Telex: 22-4944 omikk h
Telefax: 118-0109

Responsible for publication:
Mr Lajos Jánszky

Készült
az OMIKK nyomdaüzemében
(Budapest I., Gyorskocsi u. 5-7.)
Felelős vezető Tóth Károly

CONTENTS

FOLIA BIOTECHNOLOGICA

FOLIA BIOTECHNOLOGICA No. 38 1990

THEORY OF VEGETATIVE MICROPROPAGATION

author: L. Heszky

Methods of sterile vegetative micropropagation are practised in all over the world. The main technique of the propagation is the culture of meristems and sprouts by which one can obtain propagation of unlimited quantity, in theory. The process of elimination of viruses, diagnostics of viruses in plants and long-term storage of pathogen-free cultures - all are based on in vitro cultivation.

*

FOLIA BIOTECHNOLOGICA No. 39 1990

VEGETATIVE MICROPROPAGATION IN PRACTICE

author: Annamária Mészáros

This study deals with the practical steps of in vitro micropropagation of plants. Possibilities of application of this method in the mass production and improvement of plants. Laboratory for micropropagation , the necessary instruments, rules of sterile technique and the environmental conditions.

*

FOLIA BIOTECHNOLOGICA No. 40 1990

EMBRYO TRANSFER IN SHEEP
author: S. Cseh

The purpose of this study is to give a survey on the methods of sheep embryo transfer on the basis of specialised literature and our experience gained by transfer of 2000 embryos at Üllö. During the exposition we had in view the requirements of the practical application of the method.

*

FOLIA BIOTECHNOLOGICA No. 41-42 1990
authors: J. König, P. Rommel

This modern process gives a number of new possibilities for the breeder: increase of quantity of breeding metarial, estimation of breeding value, novel methods of selection, export-import of embryos and their long-term storage by deep-freezing. Embryo transfer helps in solving the problems of infertility and in enhancing the efficiency of stock-breeding and of production of animal products.

*

FOLIA BIOTECHNOLOGICA No. 43-44 1990
authors: M. Hideg, J. Zsirai

The first part of this publication deals with the types of synthetic membranes and the basic principles of separation and with the advantages and disadvantages of mebrane systems of different configuration. There is a special chapter about the main relations of membrane transport processes and the membrane models. The monograph outlines the theoretical base and processes of the most important procedures of membrane preparation. Gives a survey of the main trends in membrane qualification and gives advice to the user about the choice of membranes.

It demonstrates all the possibilities of application, for example in the processing of natural waters, in the food industry, in the protection of the environment and in the biotechnology.

There is a special chapter on the gas separation, pervaporation and electrical dialysis. Special attention is payed to the economical side of the application fields.

FOLIA BIOTECHNOLOGICA No. 45 1990
authors: P. Balogh, Zsuzsa Bebök, Timea Berki, P. Németh, Gy. Szekeres

In the realm of biotechnology, medical biotechnology is gaining more and more importance - due to research and development results of recent years. Primarily, molecular immunobiological and immunogenetical activities, which can influence the methodology and attitude of many other, seemingly remote fields of interest. Considering only the hybridoma technology or the new possibilities of immunomorfology, one can see that these permit to realize research work with extensive applications and giving new information and which, apart from human health care diagnostics and treatment processes are of importance in veterinary hygiene and stock breeding and other fields of agriculture as well, not mentioning other applications.

This publication has been complied with the non-hidden intention to give an insight to young experts and scientist, university and high school students into immunology, and make them interested in this field which medical scientist cannot (and do not) want to monopolize.

*

NAPJAINK BIOTECHNOLÓGIÁJA No. 23 1990

HAZARD IN BIOTECHNOLOGY
author: J. Csorba

Experience on biotechnological regulatory policy and safety concerns. The main topics of the publication are: the risk related to the high technology, the regulatory policy in biotechnology of the USA, change and development of the European regulation considerations and the safety of biotechnological research and industrial units.

*

NAPJAINK BIOTECHNOLÓGIÁJA No.24 1990

5th ROUND-TABLE CONFERENCE ON ANIMAL BIOTECHNOLOGY
ed: J. Seregi, J. Gaszler, L. Kállai

Papers of the conference are given in Hungarian and one lecture in the English language.

*

NAPJAINK BIOTECHNOLÓGIÁJA No.25 1990

RECENT DEVELOPMENTS IN BIOTECHNOLOGY APPLIED TO AGRICULTURE AND FOOD INDUSTRY

The proceedings of the joint seminar of the Federation of Technical and Scientific Societies of Hungary and the American Association for the Advancement of Science, 16-20 May, 1988, Budapest (in English).

NAPJAINK BIOTECHNOLÓGIÁJA No. 26 1990

3rd BIOTECHNOLOGICAL AND 2nd NATIONAL CONFERENCE ON MEMBRANE TECHNOLOGY, COUNTY KOMÁROM, NYERGESÚJFALU, 18 OCTOBER, 1989.
ed. P. Fábry

The papers of the conference present the research and development work on the field and the application of the results in the industry.

*

NAPJAINK BIOTECHNOLÓGIÁJA No. 27 1990

5th SCIENTIFIC SYMPOSIUM OF SOCIALIST COUNTRIES ON BIOTECHNOLOGY, 4-8 SEPTEMBER 1989, BALATONSZÉPLAK

The publication contains four papers in English from the material of the symposium. The titles are the following: Cellulose degradation, Mineral biotechnology: current state and future prospects, Some achievements of biotechnology application in anticancer drug research in China, Live recombinant vaccines for humans.

*

MICROORGANISM COLLECTION

SERVICES PROVIDED BY THE NATIONAL AGRICULTURAL AND INDUSTRIAL MICROORGANISM COLLECTION
\J.Lehoczky-Tornai\
Élelmezési Ipar, 44 (11)| 405-407, 1990.

The National Collection of the Agricultural and Industrial Microorganisms is operating as an international depository organ since June 1, 1986. At present our phylum collection maintains 410 saccharomyces, 125 moulds and 280 microbe strains. The number of our patented cultivations is around 300. Our collection is a member of the European Organization of Phylum Collection and of the World Federation of Phylum Collections.
Our Collection provides the following services:

- procurement and marketing of phyla,
- preservation in job work, secured storage,
- identification of bacteria, moulds and saccharomyces, determination of G+C mol%, DNS homology examination,
- computerized identification,
- search in the data bases of foreign phylum collections by direct computer connection,
- patent depositing and preservation of phyla requiring national and international protection,
- organization of specialized courses, controlling postgraduate individual advanced education, provision of expert advice.

GENETIC ENGINEERING

SIZE-DEPENDENT REGENERATION OF GIBBERELLA PROTOPLASTS
\CS.Vágvölgyi, B.Brückner, A.Frankó\
Acta Microbiologica Hungarica, 37 (4)| 375-378, 1990.

Regeneration ratios of protoplasts formed from the plant pathogenic Gibberella fujikuroi with a mixture of lytic enzymes were studied. The heterogenous population of protoplasts was separated into groups differing in regeneration ratio. The frequency of regeneration was higher for large protoplasts containing an increasing number of nuclei.

SEQUENCES UPSTREAM OF THE -35 HEXAMER OF rrnB P1 AFFECT PROMOTER STRENGHT AND UPSTREAM ACTIVATION
\A.C. Josaitis, T.Gaal, W.Ross, R.L.Gourse\
Biochimica et Biophysica Acta, 1050:307-311, 1990.

Transcription from Escherichia coli ribosomal RNA promoters is increased about 20-fold in vivo by a DNA sequence (the Upstream Activation Region, UAR)located upstream off the -35 conserved hexamer. The UAR stimulates transcription through two mechanisms: one which involves binding of the Fis protein to the UAR, and another mechanism which functions in the absence of additional protein factors. We have previously constructed a collection of mutations in the region upstream of the -35 hexamer of rrnB P1. Most of these mutations have either no effect on promoter activity or decrease activity 2-5-fold in vivo (Gaal,T., Barkei,J.,Dickson, R.R., De Boer, H.A., De Haseth, P.L., Alavi, H. and Gourse, R.L.(1989) J.Bacteriol. 171,4852-4861). Two mutations leave both the -35 consensus hexamer and the Fis binding consensus sequence intact, yet have larger (14-50-fold) effects on transcription. One substitution just upstream of the -35 hexamer (a C to T change at position -37) primarily affects intrinsic promoter strenght, leaving the UAR functional. On the other hand, a three base pair deletion (bases -38 through -40) severely reduces UAR-mediated activity. A substitution covering the three base pair deletion was constructed and found to be activated normally. UAR function appears

dependent on its position relative to the RNA polymerase binding site, suggesting that a particular spatial geometry may be necessary for Fis- dependent and\or factor-independent activation to occur.

REGULATORY ELEMENTS DOWN STREAM OF THE PROMOTER OF AN rRNA GENE OF E. COLI
\Katalin,Csiszár, T.Lukacsovich, P.Venetianer\
Biochimica et Biophisyca Acta, 1050: 312-316, 1990.

Previously we have shown that plasmid constructs carrying a reporter gene fused to the P_2 promoter of the E. coli rrnB gene exhibited a strange two-phase kinetics of expression depending on the physiological conditions of the cell if a short DNA region downstream of the promoter was present between the promoter and the reporter gene (Lukacsovich et al. 1987. J.Bacteriol. 169, 272-277). Insertion of a synthetic oligonucleotide corresponding to the first half of this region into constructs where the reporter directly follows the promoter, leads to a complete block of expression in vivo, while in vitro - in a purified system - transcription is not inhibited. Band-shift experiments indicate that the putative regulatory region downstream of the promoter specifically binds protein(s) present in total bacterial extracts.

ENDONUCLEASE-FREE, PROTOPLAST-FORMING ENZYME PREPARATION AND ITS APPLICATION IN FUNGAL TRANSFORMATION
\M. Mink, H.J. Höltke, C. Kessler, L. Ferenczy\
Enzyme microbiology and Technology, 12: 612-615, 1990

An endonuclease-free, protoplast-forming enzyme complex was prepared from the "snail enzyme". The purified preparation has high protoplast-forming activity comparable to the crude enzyme complex without destroying circular plasmid DNA. Furthermore, a higher transformation rate was achieved by the application of the endonuclease-free enzyme complex in both yeast and filamentous fungal vector-host system.

PHAGES AND PLASMIDS OF BACILLUS LICHENIFORMIS AS POTENTIAL CLONING-VECTORS
\A.Holczinger- Z.Prágai, L.Székely, M. Kovács, T.Sík\
5th European Congress on Biotechnology,Copenhagen 1990. Poceedings p.173.

The fermentative production of Bacitracin with B.licheniformis is frequently disturbed by the lytic action of bacteriophages. This might be due to induction of lysogenes or infection by virulent phages. As part of strain improvement program stable lysogeny has to be maintained or resistance against virulent phages established, occasionally both. The industrial B.licheniformis 138 strain was shown to be triple lysogenic.

After spontaneous or Mitomycin C induction the phages were identified and morphologically characterized by electron microscopy. Two of the phages were identical with the already described temperate LP 52 and the defective DLP 10716 . The third new type was colled BLF with polyhedral head (76 x 70 nm) and unusual long, flexible tail (355 nm). Suitable bacteria were chosen from Strain Collections to distinguish infection specificity of LP 52 and BLF. They were also different serologically and in their endonuclease fragment patterns, but LP52 was identical with the published one.

The spontaneous lysis of the triple lysogenic strain B.licheniformis 138 was 10^{-2} - 10^{-3}. Phage BLF could be eliminated by mutagenic treatment, but the remaining double lysogenic bacteria, carrying LP 52 and DLP 10716 phages were less stable with 10^{-1} spontaneous induction, less suitable for fermentation. Restored triple lysogeny regained stability. BLF decreased spontaneous induction in other B.licheniformis lysogenes, too.

From some lysed industrial samples a virulent phage BL 11 was also isolated and characterized morphologically, serologically, according to their infection specificity and endonuclease fragment pattern. This phage was different from the described Theta phage. The industrial producer strain was sensitive to BL 11 phage. The selected spontaneously resistant, surface receptor mutant bacteria were unstable. Internal immunity against BL 11 infection was therefore introduced to B.1.138 from a bacitracin non producing but phage immune B.1.XV (ATCC 9800) strain by protoplast fusion. Suitable selection resulted immune derivative with even increased productivity.

Phage BLF was performing generallized transduction. Close link age between arg and trp was determined in one strain and ala, thr, hom in another.

BLF phage DNA does not have recognition sites for HinD III and EcoR I endonucleases. Introducing these sites and antibiotic resistance, suitable cloning vector can be formed. Transformation of protoplasts with plasmids pUB110 (4,5 kb) with Km,Ph1 and pTV1 (12,4 kb) with Cm was the other succesful genetic information transfer in B.1. 138. The later proved to be useful to introduce Tn917 with MLS resistance to the bacterial genome. Optimizing the regeneration of protoplasts in saccharose, selection was achieved with lower concentration of antibiotics and giving higher frequency of transformation.

Cryptic plasmides were found in natural isolates of B.licheniformis. Two main sizes were 40-60 and 6-7 kb respectively, not too different in various combinations. Two labeling methods were attempted, to form cointegrates with pUB110 or to transfer the 1,5 kb Alu I fragment with the resistence block to the cryptic plasmides in order to construct suitable cloning vectors.

ADP-RIBOSYLATION OF MEMBRANE PROTEINS OF STREPTOMYCES GRISEUS STRAIN 52-1

\A.Penyige, Gy.Barabás, I.Szabó, J.C.Ensign\

FEMS Microbiology Letters, 69: 293-298, 1990.

Membranes purified from cells of Streptomyces griseus strain 52-1 possess an ADP-ribosyltransferase activity. The enzyme transfers the ADP-ribose moiety of NAD to one major membrane protein of Mr! 32000 and 2-3 minor proteins of larger molecular weights. The effects of inhibitors on the ADP-ribosyltransferase activity proves that the reaction is enzymatic and suggests that the enzyme ADP-ribosylates the guanidine group of arginine. The kinetics of liberation of ADP-ribose during alkaline hydrolysis of the modified proteins is consistent with the arginine-ADP- ribose bond. This is the first report of ADP-ribosylation of proteins in a Gram-positive bacterium.

NUCLEOTIDE SEQUENCE OF THE PUTATIVE REGULATORY GENE AND MAJOR PROMOTER REGION OF THE STREPTOMYCES GRISEUS GLYCEROL OPERON
\A.Bolotin, S.Bíró\
Gene, 87: 151-152, 1990.

Nucleotide sequencing of the deduced major promoter region of the glycerol utilization operon and an upstream regulatory gene of Streptomyces griseus reveals extensive similarity to the previously sequenced homologous S.coelicolor region (Smith and Chater, J.Mol.Biol. 204 (1988) 569-580). However, regions showing extensive divergence are found in the noncoding parts of the sequence. These may help to evaluate the significance of various sequence features in relation to promoter activity.

A PHOSPHATE GROUP AT THE cos ENDS OF PHAGE LAMBDA DNA IS NOT A PREREQUISITE FOR IN VITRO PACKAGING: AN ALTERNATIVE METHOD FOR CONSTRUCTING GENOMIC LIBRARIES USING A NEW PHASMID VECTOR, λ pGY97
\Éva Vincze, Gy.Kiss\
Gene, 96: 17-22, 1990.

It is shown here that the phosphate groups at the cos ends of phage lambda - DNA are not a prerequisite for in vitro packaging. Molecules with phosphatase-treated cos ends are packaged in vitro as efficiently as native lamda-DNA. This observation can be used for an alternative strategy to improve the efficiency of gene library construction, since cos-cos ligation decreases in vitro encapsidation and infectivity. Dephosphorylated cos ends and a new phasmid vector lambda-pGY97 have been used to construct a representative gene bank of alfalfa in a Mcr^{-1} (5-methylcystosine restriction deficient) Escherichia coli host strain. These recombinant clones can be propagated as phages or more conveniently as plasmid in $recA^{-1}$ E.coli to prevent possible homologous recombination events between repetitive sequences of the insert that would otherwise interfere with clone stability. The 5-19 kb inserts can be easily recloned as plasmids from the recombinant phasmids with simple EcoRI digestion and re-ligation. This observation also implies that the construction of gene libraries in cosmid vectors can be made more efficient if cos-cos ligates were cleaved by lam-

da-terminase just before in vitro packaging.

FORMATION, MUTATION, REGENERATION AND FUSION OF PROTOPLASTS FROM GIBBERELLA FUJIKUROI
\B.Brückner, K.Bermuc:ɜz, A.Frankó\
4th Int.Mycological Congress, Regensburg 1990.

Considerable interest has been focused on the isolation of fungal protoplasts and the use of protoplast technique for strain improvement. The protoplast fusion is a viable alternative to traditional autogenetic treatment.
Strain improvement programs routinely include protoplast fusion between different mutant lines. Prerequisite for fusion experiments is the preparation of a high yield of protoplasts from fungi in a short time using either commercially available or laboratory prepared enzyme preparations and the isolation of genetically marked mutant strains in order to monitor fusion processes. This paper demonstrates a procedure for isolation and regeneration of protoplasts from mycelium of the gibberellin producing fungus Gibberella fujikuroi. Furthermore, some genetic techniques to isolate genetically marked mutants of Gibberella fujikuroi are described. The auxotrophic, pigment and pigmentless albino mutants could be selected after UV and MNNG mutagenesis of microconidia and protoplasts. In general, auxotrophic strains were recovered following filtration enrichment. The productivity of all mutants was determined and the high yielding gibberellin producers were used for several fusion experiments. Heterokaryons were formed following protoplast fusion of different auxotrophic strains and of albinostrains, blocked in different steps of carotenoid synthesis. Because of the instability of heterokaryons the colonies were transfered to minimalagar supplemented with campher as a diploidization agent or MBC as haploidization agent. Fast growing colonies or sectors were isolated and gibberellin productivity was determined. Therefore, we could demonstre that the methods of mutagenesis and fusion of protoplasts can be used for strain improvement of Gibberella fujikuroi.

ELECTROTRANSFORMATION OF ASPERGILLUS NIDULANS PROTOPLAST SUBCLASSES
\A.Frankó, Cs.Vágvölgyi\
Int. Mycological Congress, Regensburg 1990.

Protoplast of Aspergillus nidulans G 191 were separated by rate zonal centrifugation on Nycodenz gradient into groups differing in diameter. Plasmid pKTM7 was grown in E.coli HB 101 and prepared by alkaline extraction.
The optimal parameters of electrotransformation were determined empirically for each group of protoplast, because of the value of high-voltage electric field permeabilizes the protoplast membrane depends on the diameter of protoplasts.
Experiments have been performed in low ionic strength media, an alternating current was applied to induce the adsorption of plasmid to the surface of protoplasts and membrane-membra-

ne contacts to harbour them. The transformation frequency was higher for large protoplast containing an increased number of nuclei.

EXPERIMENTAL DEMONSTRATION OF PARASEXUAL CYCLE OF ASPERGILLUS CARBONARIUS
\F.Kevei\
4th Int. Mycological Congress, Regensburg 1990.

Incompability studies amongst black Aspergilli need precise definition in differences of species. A. carbonarius (Thom) is the most distinct member within the A. niger species group possessing multi-nucleated conidial system. Conidia of wild type strain resulted in morphological mutants following various mutagenic treatment. These mutants exhibit different growth rate, either reduced or more abundant conidial structure and few of them possess colour markers as fawn or white. In second step mutagenic treatment fawn and white coloured mutants resulted in a series of stable auxotrophs. Complementing auxotroph characters associated with different colours were pairing in parasexual crosses formed heterokaryons with wild type morphology. The crosses were carried out either by hyphal anastomosis or protoplast fusion. Conidia formed on heterokaryons showed parental conidial segregation but a large proportion of them remained heterokaryotic because of their multinucleated character. In very rare cases, a new, more stable colony type similar to wild morphology can be obtained from conidia of heterokaryons. The new phenotype proved to be diploid showing no parental segregation of conidia, presence of half number of nuclei in the same cytoplasmic volume and doubled DNA amount per nucleus. When protoplasts derived from young heterokaryotic colonies were regenerated on minimal medium, diploids can be obtained more frequently. Spontaneous and benomyl induced segregation of diploid resulted in a wide range of parental characters in haploid segregants.

CHARACTERIZATION OF THE KILLER PHENOTYPE OF SACCHAROMYCES DAIRENSIS CBS 421
\J.Kucsera\
4th Int. Mycological Congress, Regensburg 1990.

Killer phenotype examination of Saccharomyces species from CBS collection resulted in the finding that S. dairensis CBS 421 has killing activity. Its ability to kill other yeasts was tested among the members of Saccharomyces genus and in other genera. The optimal pH of the toxin action was in the range of 4,5-5,2. Inactivation of the toxin could be achieved by proteolytic enzymes at pH 4,2. Curing of the killer trait by growth at elevated temperature or in the presence of sublethal concentrations of cycloheximide, ethidium bromide or acriflavine did not result in the elimination of the factor. UV treatment at 75-90% survival rate proved to be inefficient too. Attempts were made to isolate dsRNA or DNA plasmids from total cellular nucleic acids, crosses were induced between killer and sensitive strains and the

progenies were analysed. All results show that S. dairensis CBS 421 killer character is different from the Saccharomyces types published so far.

ANALYSIS OF A HYBRID OF ASPERGILLUS NIDULANS AND ASPERGILLUS QUADRILINEATUS
\J.Varga, J.H.Croft\
4th Int. Mycological Congress, Regensburg 1990.

An interspecific hybrid produced by polyethylene glycol induced fusion of protoplasts of an Aspergillus nidulans master strain and an Aspergillus quadrilineatus auxotrophic mutant was treated with various compounds, known to have haploidizing effect on A. nidulans diploids (acridine yellow, benomyl, carbon tetrachloride, chloral hydrate, ethanol, griseofulvin, methyl thiophanate, sodium deoxycholate, miconazole, m-, o- and p- fluorophenylalanine). Haploid segregants were selected and further analysed. We could not observe any significant differences between the segregation patterns obtained with different segregating agents. Analysis of the segregants showed that the distribution of the A. nidulans linkage groups were random. Linked segregation of any markers was not observed. Significantly higher frequencies of parental marker combinations were detected in some cases, but the nonparental types were in majority in other cases. These results confirm that the two parents are closely related taxonomically, and suggest a high degree of homology between their chromosomal DNAs.

TRANSFER OF ISOLATED NUCLEI INTO FUNGAL PROTOPLASTS
\Cs. Vágvölgyi, L.Ferenczy\
4th Int. Mycological Congress, Regensburg 1990.

Transmission of isolated Aspergillus nidulans nuclei via protoplast fusion was carried out. Intact nuclei were isolated from protoplasts produced in high amounts from young mycelia grown in liquid culture. For final purification of the crude nuclear fraction a Nycodenz density gradient centrifugation was applied. The resulting nuclei were used for caryoduction. A polyethylene glycol - Ca^{2+} - dimethylsulphoxide system was used to induce the uptake. Complemented heterokaryotic colonies with a frequency of 5×10^{-7}-10^{-8} were observed.

SEPARATION OF CHROMOSOMES FROM MUCOR CIRCINELLOIDES
\Cs. Vágvölgyi, L. Manczinger\
4th Int. Mycological Congress, Regensburg 1990.

An electrophoretic karyotype of Mucor circinelloides f. lusitanicus has been obtained using orthogonal field alternation gel electrophoresis (OFAGE). Five distinct chromosomal mobility groups were observed. Using the chromosomes of Saccharomyces pombe as size standards, the sizes of Mucor chromosomes found to be between 4500 and 8000 kilobase pairs with a total genome size of approximately 30 000 kilobase pairs.

RHIZOBIUM MELILOTI LIPOPOLYSACCHARIDE AND EXOPOLYSACCHARIDE CAN HAVE THE SAME FUNCTION IN THE PLANT-BACTERIUM INTERACTION

\P. Putnoky, Gy. Petrovics, A. Kereszt, E. Grosskopf, D.C.Ha, Zsófia Bánfalvi, A. Kondorosi\
Journal of Bacteriology, Sept. 1990. 5450-5458.

A fix region of Rhizobium meliloti 41 involved both in symbiotic nodule development and in the adsorption of batriophage 16-3 was delimited by directed Tn5 mutagenesis. Mutations in this DNA region were assigned to four complementation units and were mapped close to the pyr-1 and pyr-29 chromosomal markers. Phage inactivation studies with bacterial cell envelope preparations and crude lipopolysaccharides (LPS) as well as preliminary characterization of LPS in the mutants indicated that these genes are involved in the synthesis of a strain-specific LPS. Mutations in this DNA region resulted in a Fix^- phenotype in AK631, an exopolysaccharide (EPS)-efficient derivative of R. meliloti 41; however, they did not influence the symbiotic efficiency of the parent strain. An exo region able to restore the EPS production of AK631 was isolated and shown to be homologous to the exoB region of R. meliloti SU47. By generating double mutants, we demonstrated that exo and lps genes determine similar functions in the course of nodule development, suggesting that EPS and LPS may provide equivalent information for the host plant.

DETECTION OF A 2 μ DERIVATIVE YEAST PLASMID WITH ALTERED PROPERTIES

\M. Mink, J. Stülke, K. Büttner\
Journal of Basic Microbiology, 30 (7)| 529-534, 1990.

The Saccharomyces cerevisiae strain RXII, like many others, harbours plasmid DNAs and one category of them is homologous to the 2 μ plasmid of yeast. DNA-DNA hybridization experiments indicated altered structures of this species as regards the number and distribution of the restriction sites. The efforts made to clone either the whole plasmid in pBR328 or its fragments in pBR322 vectors remained unsuccesful, since deleted plasmids were isolated without insert DNA, end even the loss of vector sequences was observed. The data suggest, that the 2 μ derivative plasmid in strain RXII represent an unique category of this extrachromosomal genetic element.

NEW APPROACHES TO INCREASE THE EXPRESSION AND STABILLITY OF CLONED FOREIGN GENES IN ESCHERICHIA COLI

\T. Lukasovich, Gabriella Balikó, A. Orosz, Éva Balla, P. Venetianer\
Journal of Biotechnology, 13: 243-250, 1990.

A family of expression plasmid vectors were constructed by fusing the strong P^2 promoter of the rrnB gene of Escherichia coli (coding for ribosomal RNA) to the lac operator, thereby eliminating regulatory sequences from the rrnB gene and placing the expression under lac

repressor control. This promoter proved to be stronger in vivo than the well-known consensus tac promoter, and its strenght could be further increased by converting the sequence to consensus. The stability of the recombinant proteins could be increased by fusion to various lenghts of the N-terminal end of beta-galactosidase, or by inserting a synthetic oligonucleotide, coding for heptathreonine.
A new method was developed for the stabilization of recombinant plasmids without antibiotic selection, based on the presence of an essential gene on the plasmid and its absence from the example of a plasmid expressing human proinsulin.

A FAMILY OF EXPRESSION VECTORS BASED ON THE rrnB P_2 PROMOTER OF ESCHERICHIA COLI
\T. Lukacsovich, A. Orosz, Gabriella Balikó, P. Venetianer\
Journal of Biotechnology, 16: 49-56, 1990.

We describe here the construction of a family of expression vectors, based on the P_2 promoter of the Escherichia coli rrnB gene by removing regulatory sequences downstream of the Pribnow-box and replacing them with the lac operator. These vectors allow cloning of foreign genes in such a way that their products are synthesized either in the form of fusion proteins of different lenghth, or without fusion partners, with or without the original translational initiation signals. One of the vectors contains a synthetic oligothreonine-coding sequence that helps to stabilize the product of the cloned gene. These vectors allow high-level regulated expression of foreign genes, even if their products are relatively short peptides.

IMMUNOFLUORESCENCE DETECTION OF NUCLEAR ANTIGENS IN SUPRAMOLECULAR CHROMATIN SPREADS
\J. Schlammadinger\
Methods in Molecular and Cellular Biology, 1 (516)| 235-241, 1990.

A new method for isotonic lysis of mammalian cells and supramolecular spreading of nuclear material is described. The suitability of the resulting chromatin spreads for the immunofluorescence microscopic (IFM) detection of certain classes of anti-nuclear autoantibodies (ANA) is shown. This new technique provides not only a convenient alternative to the traditional substrates already applied for IFM diagnostics, but it also holds promises of the development of high resolution in situ nucleic acid hybridization procedures.

GENE TRANSFER BY PROTOPLAST FUSION FOR FUNGAL STRAIN IMPROVEMENT
\F. Kevei, L. Ferenczy\
Napjaink Biotechnológiája, 25: 58-67, 1990.

This paper describes the most important steps and contributions made by our group in the field of protoplast research. We do not intend to review all the results in this field, these were recently surveyed by Peterdy and Ferenczy (1985).

Our group has been working on fungal protoplast system for nearly twenty years. Since the early seventieth, the aim of our protoplast research has been to develop protoplast fusion system and to apply this technique for various fusion systems and to apply this technique for various gene transfer purposes. A prerequisite of using protoplasts for fusion was to have experience in the manipulation of yeast and fungal cells, and to establish optimum conditions for protoplast release and regeneration. A wide range of yeast species (Kevei et al. 1972) and filamentous fungi (Ferenczy et al. 1970, 1974, 1975a, Pesti and Ferenczy 1979) were studied.

RHIZOBIUM PHAGES IN HOST-PHAGE INTERACTIONS, GENETIC INFORMATION TRANSFER AND STRAIN CHARACTERIZATION
\T.Sík\
Nitrogenfix '90 Conference, Poznan, May 7-10. 1990.

HOST-SPECIFIC CHEMOTAXIS OF RHIZOBIUM MELILOTI
\É. Kárpáti\
Nitrogenfix '90 Conference, Poznan, May 7-10. 1990.

CHEMOTACTIC ATTRACTANTS FROM LEGUMINOUS PLANTS
\T. Ponyi\
Nitrogenfix '90 Conference, Poznan, May 7-10. 1990.

CLONING AND NUCLEOTIDE SEQUENCE OF THE GENE ENCODING THE Eca1 DNA METHYLTRANSFERASE
\V. Brenner, P. Venetianer, A. Kiss\
Nucleic Acids Research, 18: 355-359, 1990.

The gene coding for the GGTNACC specific Eca1 DNA methyltransferase (M. Eca1) has been cloned in E. coli from Enterobacter cloacae and its nucleotide sequence has been determined. The eca1M gene codes for a protein of 452 amino acids (M_r: 51 111). It was determined that M. Eca1 is an adenine methyltransferase. M.Eca1 shows limited amino acid sequence similarity to other adenine methyltransferases. A clone that expresses Eca1 methyltransferase at high level was constructed.

CLONING AND NUCLEOTIDE SEQUENCE OF THE GENES CODING FOR THE Sau96I RESTRICTION AND MODIFICATION ENZYMES
\L. Szilák, P. Venetianer, A. Kiss\
Nucleic Acids Research, 18: 4659-4664, 1990.

The genes coding for the GGNCC specific Sau96I restriction and modification enzymes were cloned and expressed in E. coli. The DNA sequence predicts a 430 amino acid protein (M_r: 49252) for the methyltransferase and a 261 amino acid protein (M_r: 30 486) for the endonuclease. No protein sequence similarity was detected between the Sau96I methyltransferase and endonuclease. The characteristic for m^5C-methyltransferases. In addition to this, M.Sau96I shows similarity, also in the variable region, with one m^5C-methyltransferase (M.SinI) which has closely related recognition specificity

(CGA\TCC). M.Sau96I methylates the internal cytosine within the GGNCC recognition sequence. The Sau96I endonuclease appears to act a monomer.

THE NUCLEOTIDE SEQUENCE OF A NODULE-SPECIFIC GENE, Nms-25 OF MEDICAGO SATIVA: ITS PRIMARY EVOLUTION VIA EXON-SHUFFLING AND RETROTRANSPOSON-MEDIATED DNA REARRANGEMENTS

\Z. Végh, Éva Vincze, R. Kadirov, G. Tóth, Gy. B. Kiss\

Plant Nuclear Biology, 15: 295-306, 1990.

We present the primary structure of a nodule-specific gene, Nms- 25 from Medicago sativa L. cultivar Nagyszénási. Analysis of the nucleotide sequence of Nms-25 revealed that this gene shows all the characteristics of an interrupted plant gene consisting of 13 exons and 12 introns. The promoter region of Nms-25 contains the common promoter elements of plant genes as well as motifs which are supposed to be involved in nodule-specific expression. There are two exon-like sequences in the gene named PE1 and PE2 which are not present in the sDNA clones of Medicago sativa cultivar Cardinal. Intron 9 carries a retrotransposone-like element, Tms1, which might be responsible for downstream deletion events in which a heptanucleotide, ATTAGCT, might have been involved. Most of the exons, exept 1, 12 and 13, are similar to each other both in lenght (54bp) and sequence (up to 94% sequence similarity). All exons are interrupted by introns in the same phase (type I). It is suggested that exon-shuffling based on illegitimate recombination in which the ATTAGCT motif might have played an active role, and retrotransposon-mediated DNA rearrangements were the primary events in the molecular evolution of the Nms-25 gene.

IDENTIFICATION AND cDNA CLONING OF A NEW NODULE--SPECIFIC GENE, nMS-25 (NODULIN-25) OF MEDICAGO SATIVA

\Gy.B. Kiss, Éva Vincze, Z. Végh, G. Tóth, J. Soós\

Plant Molecular Biology, 14: 467-475, 1990.

A new nodule-specific gen, Nms-25 (nodulin-25), was identified in cDNA clones isolated from a nodule-specific cDNA library of Medicago sativa. The first transcript of this gene appeared 9 days after inoculation of the roots with Rhizobium meliloti. The time of expression and the quantity of the transcripts of the Nms-25 gene was similar to that of leghemoglobin genes suggesting a similar regulation. A protein of 246 amino acids could be deduced from a full-length cDNA clone. The first 24 amino acids at the N-terminal end of this protein formed a signal sequence which might direct membrane transport into the peribacteroid space. Using different predictive methods the signal sequence cleaved protein was tentatively predicted to be a water-soluble enzyme, but not hydrolase.

GUANOSINE 3'-DIPHOSPHATE 5'-DIPHOSPHATE IS NOT REQUIRED FOR GROWTH RATE-DEPENDENT CONTROL OF rRNA SYNTHESIS IN ESCHERICHIA COLI

\T. Gaál, R.L. Gourse\

Proceedings of the National Academy of Sciences, USA, 87: 5533- 5537, July 1990.

rRNA synthesis in Escherichia coli is subject to at least two regulation systems, growth rate-dependent control and stringent control. The inverse correlation between rRNA synthesis rates and guanosine 3'-diphosphate 5'-diphosphate (ppGpp) levels under various physiological conditions has led to the supposition that ppGpp is the mediator of both control mechanisms by inhibiting transcription from rrn P1 promoters. Recently, relA^{-1} spoT^{-} strains have been constructed in which both ppGpp synthesis pathways most likely have been removed (M. Cashel, personal communication). We have confirmed that such strains produce no detectable ppGpp and therefore offer a direct means for testing the involement of ppGpp in the regulation of rRNA synthesis in vivo. Stringent control was determined by measurement of rRNA synthesis after amino acid starvation while growth rate control was determined by measurement of rRNA synthesis under different nutritional conditions. As expected, the relA^{-1}spoT^{-} strain is relaxed for stringent control. However, growth rate-dependent regulation is unimpaired. These results indicate that growth rate regulation can occur in the absence of ppGpp and imply that ppGpp is not the mediator, or at least not the sole mediator, of growth rate-dependent control. Therefore, growth rate-dependent control and stringent control may utilize different mechanisms for regulating stable RNA synthesis.

MOLECULAR GENETIC RESEARCH IN TEACHING BIOTECHNOLOGY

\T. Sík, A. Holczinger, É. Kárpáti\

Scientific Symposium of Agricultural Institutions in Gödöllő, April 1990.

For teaching biotechnology active research is necessary. Molecular genetic research systems were developed to introduce students in genetic experiments and problem solutions. Teaching staff and students are working together applying and developing DNA techniques, gene manipulations, molecular gene characterizations. From the results diploma works are written and also integrated in publications of the research project.

The projects are on the nitrogen fixation of Rhizobie and genetics of B. licheniformis. Parts of the Rhizobium project are the genetics of host plant induced chemotaxis, phage-host interaction, defective lysogeny and virulent phage exclusion, transposon transduction for gene identification.

With B. licheniformis the project consists of genetics of phage- host interaction, stable phage immunity and lysogeny, genetic information transfers, vector development for gene cloning.

The results of the projects are briefly interpreted.

GENE LIBRARY OF A BACILLUS LICHENIFORMIS BACTERIOPHAGE AND ITS USE

\A. Holczinger, Z. Prágai, M. Kovács, T. Sík\

Scientific Symposium of Agricultural Institutions in Gödöllő, April 1990.

The Bacillus licheniformis strain studied is triple lysogenic. One of the temperate phages BLF was identified and characterized in our laboratory. Cointegrate was formed with the pUB 110 Staphylococcus and pUC 19 E. coli plasmids. Gene library was constructed with the HinDIII fragments of BLF phage and the pUBUC cointegrate shuttle vector.
Clone isolated, are studied in transformed B. licheniformis for expression.
The work is intending to construct stable vectors and use temperate phage gene clones to insert selectable markers into criptic plasmids.

BIOENGINEERING

STIRRED FLUIDIZED-BED REACTOR DEVELOPED FOR LOW-DENSITY BIO-CATALYST SUPPORTS
\Cs. Sisak, L. Boross, B. Szajáni\
Biotechnology Techniques, 4: 15-20, 1990.

A multistage fluidized-bed bioreactor with mechanical impeller of special structure was constructed. The reactor was applied to carry-out of amino acid resolution by aminoacylase immobilized on AKRILEX C-100 type polyacrylamide beads of density being closed very much to density of substrate solution. Using a moderate mixing of the fluidized bed by the impeller the channel formation could be eliminated, so the bed could be stabilized hydrodynamically.
The performance of the stirred reactor was compared with that of packed-bed and traditional fluidized-bed ones. A considerably higher efficiency and stability of the new reactor was found.

RELEVANCE OF CO_2 IN FERMENTATION PROCESS SCALEUP
\Z.L. Lengyel\
Kem. Ind, 39 (12): 575-578, 1990.

Inhibitory and stimulatory effects on industrial scale aerobic fermentation by CO_2 has been demonstrated in our earlier works.
In scaleup processes however it was surprising to find sometimes better results of industrial scale fermentation than those of laboratory scale fermentation at the same oxygen supply.
Taking into consideration the different physical properties, chemical reactions and biological effects of CO_2 in the aqueous culture media there was correlation among the dissolved CO_2 content, the biological activity of the living cells, the volume and hydrostatic pressure of the fermenter.
The stimulating level of CO_2 must be maintained especially at the initial phase of the fermentation.
Thus in the case of laboratory scale fermentation it is necessary to mix CO_2 into the inlet air, or to make the fermentation under overpressure.
The correlation among dissolved CO_2, fermenter volume, scale, overpressure and the biological effects of CO_2 to the living cells, must be taken into consideration in modelling the process.

Such modelling may be used for scale-up or to design new laboratory experiments based on industrial scale fermentations.

DETERMINATION OF THE GROWTH OF GEL-ENTRAPPED MICROBIAL CELLS AT VARIOUS DEPTHS OF THE ALGINATE GEL

\L. Boross, P. Papp, B. Szajáni\

Physiology of Immobilized Cells, Proceedings of the Int. Symposium, Wageningen, 10-13 December 1989.

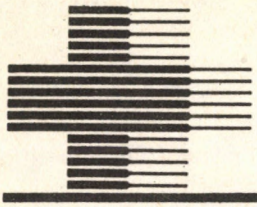
A multistep dissolution method was developed to study the growth of S. cerevisiae cells immobilized in Ca-alginate gels. The kinetics of the growth of the cells at various depths in the gel particles could be characterized by dissolution of the gel beads from layer to layer.

CRYSTALLIZATION IN BIOTECHNOLOGIES

\S. Halász, E. Bátor, T. Blickle\

Proceedings of the 11th Symposium on Industrial Crystallization, Garmisch-Partenkirchen, 18-29 September 1990.

Controlled precipitation of two antibiotics was studied by using a complete laboratory biocrystallizer unit. Results are discussed in terms of supersaturation and crystal morphology developed in pure or impure systems (fermentation liquid). Recommendation for promotion of crystal growth are also given. Conclusion: among the alternative down-stream techniques crystallization (precipitation) is still a competitive one because of its relatively low operational cost and simple apparatus. These benefits are considerable in the big-volume antibiotics production.



PHARMACOLOGY

ANTIMICROBIAL AND IMMUNOMODULATING EFFECTS OF SOME PHENOLIC GLYCOSIDES

\J. Molnár, Gy. Gunics, I. Mucsi, M. Koltai, I. Petri, Y. Shoyama, M. Matsumoto, I. Nishikoa\

Acta Microbiologica Hungarica, 36 (4): 425-432, 1989.

Several phenolic glycosides, i.e. acteoside, deshramnosyl acteoside and purpureaside A, B and C, exerted weak antibacterial effects on Escherichia coli. Acteoside had antiplasmid effects, including F'lac plasmid elimination, and inhibited kanamycin resistance transfer in E. coli. Acteoside, desrhamnosyl acteoside and purpureaside A displayed antiviral effect on Aujeszky virus. All of the phenolic glycosides decreased some human leucocyte functions., including rosette formation, nitrogen-induced blast transformation and phagocytic activity in vitro. The purpureaside C had significant proinflammatory action, however, other phenolic glycosides showed neither proinflammatory nor antiinflammatory effect on carrageenin-induced inflammation in vivo.

IN VITRO INVESTIGATION OF BK-218, A NEW ORAL AND PARENTAL CEPHALOSPORIN

\J. Szabó, J. Barabás, A. Tar, L. Kiss, M. Filep, T. Schmidt, K. Marossy, B. Tóth-Martinez, Gz. Barabás, F. Hernádi\

Antimicrobial Agents and Chemotherapy, 34 (2): Feb. 1990. 349-354.

The antibacterial activity of BK-218 was similar to that of cefamendole when it was tested against several laboratory strains. The inhibiting effect of BK-218 was greater than that of cephalexin and cefoxitin on penicillin-binding proteins of Escherichia coli HB101. This result was in close correlation with the relative inhibition of radiolabeled glucosamine incorporation (greatest with BK-218) and with the lytic effect (most intensive with BK-218). BK-218 proved to be a good inhibitor for all five of the beta-lactamases that were investigated, althought two enzymes (Enterobacter cloacae P99 and Pseudomonas aeruginosa Cilote) hydrolyzed it to some extent.

EFFECT OF AMINOGLYCOSIDE ANTIBIOTICS ON THE AUTOLYTIC ENZYME OF STREPTOMYCES GRISEUS

\I. Szabó, A. Penyige, Gy. Barabás, J. Barabás\

Archives of Microbiology, 155: 99-102, 1990.

The isolated cell wall of Streptomyces griseus 52-1 strain labelled with fluorescein isothiocyanate (FITC) and containig wall-bound autolytic enzyme was lysed as a function of different cations. The autolysis was accelerated by aminoglycoside antibiotics (streptomycin and the structurally closely related neomycin) which have a polycationic character. Since this strain is a streptomycin producer it is suggested that streptomycin may have a regulatory function on autolysis.

Autolysis very likely have other functions, too. They seem to participate in the separation of daughter cells (Fischer and Tomasz 1984) and in the formation of flagella (Fein 1979). Their role in cell wall turnover and growth is still obscure. Our aim was to investigate the effect of Streptomyces griseus which produces this antibiotic.

COMPLEX INTERACTION OF YEAST TRANSCRIPTIONAL FACTORS WITH CELLULAR AND VIRAL ENHANCERS

\J. Ghzuris, K. Polyák, I. Dencsö, E. Duda\

20th FEBS Meeting, Budapest 1990.

In vivo experiments proved that animal and viral DNA sequence of enhancer regions are able of increasing the transcriptional activity of adjacent gene in the yeast, Saccharomyces cerevisiae. Retardation electrophoresis experiments revealed the existance of yeast nuclear factors that recognize enhancer sequences with high specificity, forming stabil complexes with DNA. Further studies indicated that these factors represent a large number of proteins with molecular masses between 16 and 120 kDa. To locate the binding sites along the DNA strands we used footprinting experiments. The results indicated very complex interactions between enhancer elements and yeast nuclear proteins. In fact all enhancer motifs, recognized by known transacting proteins from animal sources were protected by yeast proteins. A purification protocol was established for the purification of the proteins and dimers and tetramers of enhancer motifs are used to identify and characterize individual yeast nuclear proteins.

YEAST PROTEASE ACTIVITY-INFLUENCED BY GLUCOSE EFFECT?

\A. Halász, M. Szakács-Dobozi, I. Szalma\

20th FEBS Meeting, Budapest 1990.

Protease activities of the investigated yeast strains S. cerevisiae, R. glutinia, and C. guilliermondi depend on growth phase, aeration rate and glucose concentration. Immunoanalytical evaluation shows that the lower protease B activity at 0,5% glucose concentration is not a result of lower protein content but caused by a reversible inactivation of the enzyme.

DETECTION AND DETERMINATION OF FACTOR C - A REGULATORY PROTEIN - IN STREPTOMYCES STRAINS BY ANTISERUM AND MONOCLONAL ANTIBODY

\F. Szeszák, S. Vitális, F. Tóth, G. Valu, J. Fachet, G. Szabó\

Archives of Microbiology, 154: 82-84, 1990.

Rabbit antisera and monoclonal antibodies were raised against factor C, a regulatory protein of Streptomyces griseus. ELISA and immunoblotting techniques suitable to determine and characterize factor C antigen in bacterial specimens were developed. Factor C antigen was detected in all the 23 Streptomyces strains and variants examined thus far and in one Bacillus subtilis too. Depending on the strain analysed it has a molecular mass of 34 000 or 70 000 in mycelial homogenates. Most of factor C was found excreted into the cultivation medium. The quantity of factor C antigen in different Streptomyces strains showed great variation. Amy^+ strains were usually good producers of factor C while Amy^- were not. This was consistent with our assumption that factor C was an inducer of reproductive phase in Streptomyces.

DISTINCTION OF FRESH AND FROZEN MEAT BY THE HADH-TEST

\F. Mietsch, R. Lásztity\

20th FEBS Meeting, Budapest 1990.

The p-hydroxyacyl-CoA-dehydrogenase (HADH) activity of beaf and pork (m.semimembranaceus) were investigated by the method of Gottesmann and Hamm (1982). On the base of the recommended limits fresh and frozen stored (-20 °C, 48 h) meat were succesfully distinguisned. Both the longer storage time and the repeated freezing and thawing enhance the release of HADH from the mitochondria. Therefore a higher HADH-activity can be measured which result in a higher reliability of the test (Hamm, Gottesmann, 1985\a. Hamm, Gottesmann, 1985\b). The authors recommand the method for routine analysis because the test is fast and reliable.

SYNERGISTIC EFFECT OF PROMETHAZINE WITH GENTAMYCIN IN FREQUENTLY RECURRING PYELONEPHRITIS

\J. Molnár, I. Haszon, T. Bodrogi, E. Martonyi, S. Turi\

International Urology and Nephrology, 22 (5)| 405-411, 1990.

Th effects of promethazine were studied in children with frequently recurring pyelonephritis which was not associated with urological abnormalities. The results of three methods of treatment were compared: 10 children were given combination of gentamycin and priomethazine for 7 days (Group I), 11 received gentamycin treatment alone for 10 days (Group II), and 19 (Group III) were on long-term oral antibiotic prophylaxis (5,6 +-!2,1 years) with episodes of intensive treatment of recurrences. In a 3-year follow-up period, the number of pyelonephritis recurrences was significantly lower in Group I than in Group II and III. Six out of 19 children in Group III had renal scarring. The authors

suggest a synergistic effect between gentamycin and promethazine therapy. Promethazine increases antibiotic sensitivity, which could contribute to the elimination of recurring urinary tract infections.

MASS SPECTROMETRIC MEASUREMENTS IN PENICILLIN FERMENTATIONS
\J. Szilágyi, K. Pólya, P. Seres\
Proc. of Ist All-Union Workshop on Mass-Spectrometric Monitoring of Fermentation Processes, Puschino, USSR, October 22-26. 1990.

A high instrumented computer controlled fermentation system was installed at the Research Department in Bioengineering of BIOGAL Pharmaceutical Works \Hungary\ to adapt and develop fermentation technologies. As a part of this system, a quadrupole mass spectrometer (QMS) measuring system, developed by ATOMKI and BIOGAL experts was connected to the fermentors and a computer. Using this multichannel and multicomponent QMS measuring system in penicillin fermentations not only the respiratory parameters of inoculum and production phase, but after sampling the chemically bound carbon-dioxide and phenil-acetic acid concentration of fermentation broth could be measured as well. With the help of our experiments and results a new conception of repeated fed batch penicillin fermentation technology was developed using the measured carbon-dioxide production rate (CPR) as a control parameter of sucrose feeding.

MASS SPECTROMETRIC MEASUREMENTS OF CO_2 METABOLISM OF ANTIBIOTIC FERMENTATIONS
\J. Szilágyi, P. Seres, K. Pólya\
Proc. of 20th FEBS Meeting, Budapest, Augustus 19-24. 1990.

CO_2 content of fermentation broth is an important parameter for studying the material and energy balance of fermentation. For monitoring respiratory parameters and controlling CO_2 production rate (CPR) a quadrupole mass spectrometer system was developed and applied in the Bioengineering Department of BIOGAL Pharmaceutical Works. Using this QMS measuring and control system we can measure not only the exhausted and physically dissolved CO_2!, but the CO_2 content of the broth chemically bound, also. Applying the developed technics we monitored the rules of the CO_2 metabolism of antibiotic fermentations. Using these results and completing them with our experiences a new sucrose feeding has been developed by controlling CPR at an optimum level presumed by us.

DEVELOPMENT OF A COMPUTER CONTROLLED PILOT PLANT IN BIOTECHNOLOGICAL RESEARCH CENTER OF BIOGAL PHARMACEUTICAL WORKS
\J. Szilágyi, P. Seres, Gy. Sántha, A. Ágoston, K. Pólya\
Proc. of 20th FEBS Meeting, Budapest, Augustus 19-24. 1990.

In 1986 a project was started in BIOGAL for the reconstruction of its fermentation pilot plant. Financially the project

was backed partly by the BIOGAL and the Ministry of Industry. Planning and implementation of the system was done by BIOGAL, VEGYTERV, VEGYÉPSZER and PROCONTROL.
The aim of the investment was to build an up-to-date bioengineering center consisting of the computer controlled 4x300 and 4x1000 litres fermenters called BIOCORD system. These fermenters are completed with 4x60 and 4x600 litres fermenters built earlier to form a multi-scale system.
A series of automated manipulations can be carried out. System is used for batch, fed-batch and repeated fed-batch technologies as well. Collection, storage and processing of data are done by both the process controlling computer and an IBM compatible PC. BIOCORD system proved to be a good tool in the research and development work of our company.

CARBON-DIOXIDE PRODUCTION AS A CONTROL PARAMETER OF REPEATED FED- BATCH PENICILLIN FERMENTATION TECHNOLOGY
\J. Szilágyi, K. Pólya, P. Seres, Gy. Sántha, K. Búzási\
Proc. of 2nd Int. Symposium ion Biochemical Engineering, Stuttgart, FRG, March 5-7. 1990.

A new technology has been developed in pilot plant scale to control repeated fed batch penicillin fermentation. In the experiments URCM type industrial Penicillium chrysogenum strain of BIOGAL was used on complex media, and the basic penicillin fermentation technology was modified according to the carbon- dioxide production rate (CPR).
CO concentration was controlled above the critical level both with mixing rate and aeration rate. pH was controlled by feeding sulphuric acid or ammonium-hydroxide. Foaming was controlled using a multichannel foam sensor and controller developed by the BIOGAL experts.
Not having on-line sensor to monitor biomass concentration, the known correlation between the biomass and CPR was used. CPR was an indicator of growth and biomass concentration. A growth curve was fitted to the measured CPR data using logarithmic linear regression to determine the forming maximum value of this parameter. This value was used to control carbon source feeding. At constant temperature, pH, top pressure and aeration rate the carbon-dioxide concentration of outlet gas could be used as the main controlling parameter.
Application of results in the practice proved to be a useful tool to develop the automatized penicillin fermentation at a higher level.

DEVELOPMENT OF STREPTOMYCES GRISEUS, THE MODE OF ACTION OF FACTOR C
\G. Szabó, S. Vitalis, F. Szeszák, I. Békési\
Proc. of the 6th Int. Symposium on Genetics of Industrial Microorganisms, Strasbourg 1990.

Streptomycetes are prokaryotic microorganisms characterized by the formation of a mycelial network. Conidium

or spore forming strains have a life cycle that starts with the germination of the conidium that brings about branching hyphae. After a period of vegetative growth morphologically distinct reproductive, aerial hyphae appear and later on the formation of conidia can be observed in them.

From a wild type Streptomyces (S.) griseus strain No. 45H a mutant designated as No. 52-1 was isolated, which did not produce conidia in submerged culture. If, however the cultivation media of strain No. 45H was added to the nonconidiating one, S. griseus No. 52-1, the formation of preconidia, charasteristic to the reproductive phase of growth was induced. The endogenous regulator - called factor C - was isolated and proved to be a protein of a molecular mass of 34 500, being active at concentrations as low as a 0,5 ng\ml^3. Later, several other compounds called bioregulators were reported, like A-factor, B- factor, Pamamycin 607 etc., (reviewed by Khokkhlov, ref.4.). In order to understand the mode action of bioregulators one may study their effect on different levels. One may ask : does it activate or repress transcription of genes, does it influence enzyme activities, does it change permeability etc., does it affect the development studied by cytomorphological methods?

A cytomorphological marker is a complex trait, it is the result of coordinated activities of enzymes. On solid medium the S. colony itself shows development to the naked eyes, first having a smooth leathery surface which is later covered by powdery layer of aerial mycelia that also may change its colour with time. The hyphae in the colony or in submerged culture differentiate into vegetative and reproductive forms but even a given hyphal segment in the same visual field is heterogenous if studied with light microscopy. This statement was true to all morphological traits studied so far , like the thickness of the cell wall, the staining of the cytoplasm, the distribution of the polysaccharides - examined with the PAS reaction - the morphology of the nucleoides stained with Feulgen reaction or methyl-green pyronine. We are aware that althought a cytomorphological trait, e.g. appearence of nucleoid is charasteristic, reproducibly recognized, two morphologically identical trait may hide differences on the biochemical level. Three questions are dealt with: How does a complex, cytomorphological trait develop in S. griseus? What relationship exists between different cytomorphological markers? How does factor C influence the development of S. griseus?

PSEUDO-PRIMYCINS BY TRANSLACTONIZATION

\Judit Frank, Gy. Dékány, I. Pelczer\

17th IUPAC Int. Symposium on the Chemistry of Natural Products, New Delhi, India, February 4-9. 1990.

Primycin, a mixture of closely related macrolide antibiotics, was treated with nucleophiles to yield in an equilibrium process water-soluble compounds with the same molecular weight as that of the starting material. Detailed high field 2D ^{1}H and ^{13}C NMR spectroscopic studies of the separated components revealed that the new compounds are

translactonized primycins, named "pseudo- primycins"formed by a unique 35---37!ring enlargement.

APPLICATION OF ENZYME REACTOR WITH AMPEROMETRIC DETECTION FOR CONTROLLING FERMENTATION PROCESS

\N. Adányi, M. Váradi\

Symposium on Bioanalytical Methods, Prague, 1990

In the field of food processing and biotechnological fermentation the quality controll, the optimization of the biotechnological processes are coming to the front.

Through the development of enzyme analytics such measuring and controlling devices, systems can be developed, which overpass the traditional sensors significantly in rapidity and simplicity.

In the course of our researches the aim was to develop a glucose sensor system connectable to a fermentor.

With the help of a home-made measuring cell we have studied the effect of the different parameters in order to elaborate an optimal measuring technologie. The role of the pH-value, the effect of the temperature, as well as the dependance of the flow velocity considering the developed enzyme reactor and amperometric measuring cell have been examined.

We have found that in case of the glucose, which is one of the most frequent substratums when speaking about fermentation processes a measuring-cell of the required sensitivity can be shaped. We used this technique for controlling the fermentation process of glucose-amylase.

FOOD INDUSTRY

CHARACTERIZATION OF PEPTIDES ENRICHED IN METHIONINE BY ENZYMATIC PEPTIDE MODIFICATION
\Gy. Hajós, H. Notzold, A. Halász, E. Ludwig\
Acta Alimentaria, 19 (1): 73-78, 1990.

Methionine enriched polypeptides were produced from an enzymatically prehydrolized milk protein and L-methionine ethyl ester hydrochloride by application of enzymatic peptide modification (EPM) method using alpha-chymotrypsin as catalyst. Methionine content of the product was more than twice as high as that of the substrate. The peptides of the EPM-product and the substrate were separated by thin-layer chromatography, and were then subjected to amino acid determination. The ratio of the polar and apolar amino acids of the peptides was found to be influenced basically by transpeptidation taking place in the EPM-reaction. Analysis of the peptides of these products also showed that methionine was incorporated mainly in those peptides containing a relatively high ratio of apolar amino acids.

PRODUCTION AND ANALYSIS OF IMPROVED ENOLOGICAL YEAST STRAINS
\Anna Maráz, T. Deák\
Biotech Forum Europe, 7 (19)| 63-66, March 1990.

A series of enological yeast strains was submitted to extrensive analysis from taxonomical, physiological and technological point of view and according to this, some of them were selected and applied in a strain improving program. Improvement was carried out by somatic hybridization (protoplast fusion) of strains, the resulted hybrids were selected under different conditions. Data concerning the enological properties of some of the hybrids are also reported.

FERMENTED FOODS IN HUMAN NUTRITION
\P.A. Biacs, Judit Beczner\
Catering and Health, 1: 225-232, 1990.

Fermentation of food has been practised since Neolithe times. It improves the flavour and keeping quality of the food,. and increases its nutritional value. It now takes place on an industrial scale

using selected starter culture. Food processing enzymes are now largely of microbial origin. Fermented milk product seem to have a beneficial effect on malignant disease. More research is required, especially on the development of starter cultures.

THE KINETICS OF MALTODEXTRIN HYDROLISIS BY DISSOLVED AND IMMOBILIZED GLUCOAMYLASE

\E. Nagy, K. Bélafi-Bakó, A. Ujhidy, Á. Hoschke\

5th European Congress on Biotechnology, Copenhagen, 1990.

The kinetics of the pretreated mitodextrin of low average degree of polymerisation ($\overline{DP} = 3\text{-}4$) has been investigated in homogenous (soluble enzyme) and heterogenous (immobilised enzyme) systems. The Michaelis-Menten kinetic equation was used for the kinetic model of the different oligosaccharide species from maltose to maltoheptose. (that means that the kinetical equation system with 7 differential equation has to be solved.) The values of Michaelis constant of oligosaccharides were determined by the method of Lineveawer-Burk. It has been measured the product distribution curves of oligosaccharides with degrees of polymerisation of 1-7. From it the values of reaction rate constants were determined by parameter estimation which was carried out by minimalization of a least-squares objective function, using cubic spline approximation. We compare the kinetical data in both of homogenous and heterogenous system. In the latter case we will discuss the role of mass transfer process on the hydrolisis. The theoretical product distribution curves are in good agreement with the experimental ones.

TECHNOLOGICAL DEVELOPMENT OF 1,3-DIHYDROXY-ACETONE FERMENTATION

\Á. Hoschke, P. Kató, G. Vereczkey, B. Janzsó\

5th European Congress on Biotechnology, Copenhagen, 1990.

1,3-dihydroxy-acetone (DHA) is a triose and the simplest ketose at the same time. Its main fields of utilization are the cosmetics industry, where it is the active agent of self-tanning cosmetics, it is a flavour- and smell-enhancing additive in the food and tobacco industry, the starting compound of organic syntheses, a basic material for plasticizer production and is also used in therapeutics. The production of DHA is accomplished by way of fermentation for the most part, using acetic acid bacteria and glycerol as a substrate. Large-scale fermentation started with its use as a self-tanning agent in cosmetics industry.

So far DHA fermentation has been carried out in the batch mode, there are only one continuous and a few immobilized cell culture experiments mentioned in the literature.

The objective of this work was the elaboration of a high- productivity fermentation technology, that eliminates the disadvantages of present-day technologies - e.g. low final DHA titer, low productivity, long fermentation time and occasionally low conversion -

and can be realized in a pilot scale, too, with minimal investment and cost.
During the first phase of the work shaken flask experiments were carried out in order to optimize the fermentation medium and its initial pH. During the second part batch fermentation followed on a laboratory scale and a technology with a final DHA titer of 194 g\dm^3 was worked out. Having established our fed- batch experiments this way, a technology with exponential feeding was elaborated making a final DHA titer value of 280 g\dm^3 realizable, with complete utilization of glycerol. In the pilot- plant scale experiments - carried out in 100- and 1000-litres fermentors - the laboratory scale results could be reproduced with the fed-batch technique. The DHA curves of the batch and fed-batch laboratory scale, as well as that of the fed-batch pilot-plant scale fermentation.
Summarizing our results it can be established, that a high- productivity fed-batch DHA fermentation technology has been elaborated with a final DHA titer of 280 g\dm^3, a conversion of 99% and a fermentation time of 45-50 hours, realizable on the pilot-plant scale, as well.

RADIOACTIVE METHIONINE INCORPORATION INTO PEPTIDE CHAINS BY ENZYMATIC MODIFICATION
\Gy. Hajós, T. Szarvas, L. Vámos-Vigyázó\
Journal of Food Biochemistry, 14: 381-394, 1990.

Enzymatic peptide modification was carried out using L-methionine-S-methyl-^{14}C methyl ester hydrochloride and L-^{3}Hmethionine ethyl ester hydrochloride in the reaction mixture. The experimental results revealed that part of the L-methionine-S-methyl-^{14}C methyl ester was bound as methionine to enzymatic hydsolysates of casein and serum albumin in the presence of alpha-chymotrypsin, interestingly in the highest molecular weight fraction of the product protein. A maximum curve was found to describe the relation between alpha-chymotripsin-induced incorporation of methionine and the ration of L-^{3}H methionine ethyl ester to protein hydrolysate. The covalent nature of amino acid incorporation was supported by SDS polyacrylamide gel electrophoresis in the presence of urea. The isoelectric focusing patterns of the products indicate that transpeptidation plays an essential role in the EPM reaction.
These experimental findings suggest that enzymatic peptide modification is a suitable method for the production of proteins of designed amino acid composition.

POSSIBILITY FOR GOVERMENT OF GENE TRANSCRIPTION IN YEAST EXPRESSION VECTORS BY ARTIFICIAL CIS-REGULATOR ELEMENTS
\J. Gyuris, L. Dencsó, K. Polyák, E. Duda\
Perspectives of Biotechnology, Int. Symposium, Hungary 1990.

The generegulation is one of the central topics of molecular biology. The process of decoding genes and synthesizing appropriate amount of gene product are complex and regulation can

occur at various steps along the pathway. Nevertheless, a major point of gene control occurs at the first step, i.e. initiation of messenger RNA synthesis. The control if initiation of transcription is achieved throught the interaction of trans-acting proteins with cis-acting DNA promoter elements. In the last years, many observations have increasingly pointed to common molecular mechanisms of transcriptional regulation in eucaryotic organisms ranging from humans to yeasts.
The enhancers are important specific cis-acting control elements which can stimulate transcription very efficiently from RNA polymerase class B promoters of viral and cellular genes.
We assumed taht yeast trans-acting proteins recognized sequences of the viral and tissue enhancers and enhancers were active in yeast cells. So, we have studied the interaction of the SV 40 virus and IgH gene enhancers with yeast nuclear extract and investigated the function of these foreign control elements in yeast cells inserted them at the 5' ends of the reporter gene. In vivo results have shown that enhancers are capable of increasing the transcriptional activity of heterologous gene (LacZ) from truncated CYC1 promoter in yeast, at least 6-30 fold.
In vitro results - gel retardatin, DNA binding to protein blots and DNAase I footprinting assays - have proved that their activity is mediated by yeast nuclear proteins.
We have detected by footprinting experiments complex interaction of yeast nuclear proteins with enhancer promoter region of SV 40.
According to the above-mentioned result it is not clear that SV 40 enhancer works very inefficiently in yeast cells compared to their activating potential in animal cells.
One of our hypotheses was that crowd of DNA-bound activator proteins - arranged in a sequence unusual and hardly usable by the yeast transcriptional machinery could not function as an efficient enhancer, the effect of ceratin regulatory units - motifs -, might even interfere with each others positive effect. Partial experiments were carried out to support this explanation using motifs of SV 40 enhancer. Our experimental results indicated that oligomers of AP-1 motif of SV 40 enhancer can stimulate yeast transcription approximately 20 to 50 fold while oligomers of cT-motif 1 to 3 fold better than whole enhancer does. Further in vivo studies coupling with in vitro experiments might lead to detailed picture of the factors which can stimulate basel transcription from yeast promoters efficiently and governed.

APPLICATION OF ENZYME REACTOR WITH AMPEROMETRIC DETECTION FOR CONTROLLING FERMENTATION PROCESS

\N. Adányi, M. Váradi\

Symposium on Bioanalytical Methods, Prague, 1990

In the field of food processing and biotechnological fermentation the quality controll, the optimization of the biotechnological processes are coming to the front.

Through the development of enzyme analytics such measuring and controlling devices, systems can be developed, which overpass the traditional sensors significantly in rapidity and simplicity.
In the course of our researches tha aim was to develop a glucose sensor system connectable to a fermentor.
With the help of a home-made measuring cell we have studied the effect of the different parameters in order to elaborate an optimal measuring technologie. The role of the pH-value, the effect of the temperature, as well as the dependance of the flow velocity considering the developed enzyme reactor and amperometric measuring cell have been examined.
We have found that in the case of glucose, which is one of the most frequent substratums when speaking about fermentation processes a measuring-cell of the required sensitivity can be shaped. We used this technique for controlling the fermentation process of glucose-amylase.

YEASTS AMONG FUNGI
\E. K. Novák\
4th Trilateral Conference on Yeasts, Sárospatak, July 24-28. 1989.

Since the original description in 1983 (Meyen) of the first representative - Saccharomyces - of the "peculiar group" of living beings the yeasts, this group "officially" was considered as a member of the fungal branch of the kingdom Plants.Practically, however, later on the differences and peculiarities of the groups such as the facultative anaerobic ethanolic fermentation type of metabolism, and the different types of budding (and the fission) as forms of vegetative reproduction (propagation) were emphasized, thus suggesting the opinion that yeasts form a foreign body in the otherwise homologous group of the fungi (at the end of the period, however, not considered further as plants but as a separate kingdom). Moreover, for about a century the yeasts as a group were considered monophyletic, the perfect forms (now teliomorphs) of which to be systematized in the class Ascomycetes, subclass Hemiascomycetidae, order Endomycetales, while their imperfects (now anamorphs) in the class Fungi Imperfecti (Deuteromycetes), subclass Blastomycetes, order Cryptococcales. About the latter, however, it was thought they have ascomycetous relations to be discovered in detail. Althought at that time, because of bullerae and sporobolomycetes, there were some suggestions on the relationship of "yeasts" or yeast-like organisms beyond the Ascomycetes, viz. to Basidiomycetes.
Although an increasing body of data was already published it was not until the early fifties that the idea of the polyphyletic nature of the group Yeasts got publicly. Wickerham's (1051, 1952) idea was picked up by Novák and Zsolt (1977) emphasizing that the group is heterogenous and the result of physiological and morpholigical conversion originating from different phases of the polygenesis of the fungi (mostly filamentous) directed by adaptations to environments (media) being liquid and rich in nutrients, moreover providing

adventage in struggle for life for ethanol producers. Thus yeast type should not be simply considered primitive, but a highly adapted form of fungi to special environments. The system published was adopted nearly entirely by Kreisel (1969) into his attempt to create a natural system on fungi. A partial survival of idea up to the present is represented by the adoption of the family Lipomyctaceae N. et Zs. by v.d.Walt, and v. Arx et v.d. Walt (1987) and the extension of the family Nematosporeaceae N. et Zs. - accepted also by Batra (1973) - by v.Arx et al. (1977) to Metschnikowiaceae Kamienski (cf. v. Arx et v.d.Walt, in Hoog et al. 1987).
As ananerobic ethanolic fermentation, a truncated type of energy yielding catabolic process, according to biochemists, is known not only with bacteria, but also with fungi (cf. e.g. fusaria, mucors). The peculiarity of this process is diminished.

USE OF YEAST BIOMASS IN FOOD PRODUCTION
\Anna, Halász, R. Lásztity\
CRC Press, Boca Raton, Ann Harbour, Boston

In the framework of this book a summary will given about the chemistry, molecular biology, production and processing of yeast biomass. The possible applications of whole yeast cells, protein isolates and autolysates (extracts) as food ingredients will be reviewed. The production of yeast based flavors, the use of native yeast for production of commercial enzyme preparations and some other applications in food production will be shortly treated in the Appendix.

YEASTS IN BIOTECHNOLOGY
\T. Deák\
Zentralblatt Mikrobiologie, 145: 327-351, 1990.

Yeasts have been exploited throughout the history of man in the production of alcoholic beverages and bread, and these processes still represent major biotechnological industries. In recent years the broad biochemical activities of yeasts have been increasingly used in new biotechnology. Yeasts have become one of the tools of genetic manipulations, and the molecular techniques which have been so stimulatory to the rapid advances in bioscenes, have also contributed to the succesful application of yeasts for the production of novel biotechnological products.
Exploitation of yeasts
Traditional biotechnology
- conversion of carbohydrates into ethanol, carbon dioxide, biomass
New biotechnology
- production of heterologous proteins
- biotransformation
- production of flavour compounds and lipids.

PLANT BREEDING

EFFECT OF DIFFERENT CRYOPROTECTANTS AND TRANSFER TEMPERATURES ON THE SURVIVAL RATE OF HEMP (CANNABIS SATIVA L.) CELL SUSPENSION IN DEEP FREEZING
\Zs. Jekkel, L.E. Heszky, A.H. All\
Acta Biologica Hungarica, 40 (1-2): 127-136, 1989.

Adequate cell dehydration is the precipitating element in the successful cryopreservation of plant cells and organs. This could be achieved by using different cooling rates, transfer temperatures and cryoprotectants.
Experiments were performed to determine these critical points in the freeze preservation procedure of Cannabis sativa (L.) suspension cultures. The explants were frozen at a cooling rate of 2 °C\min, while the transfer temperature were -10 °C, -20 °C, -30 °C, -40 °C and -50 °C. The applied cryoprotectants were the DMSO, glycerol, proline and PEG in different concentration. The highest viability (58%) was obtained by using 10% DMSO and at -10 °C transfer temperature. The optimum transfer temperature varied remarkably by different cryoprotectant concentrations indicating the importance of their interactions.

ORGAN-SPECIFIC AND PLOIDY-DEPENDENT SOMACLONAL VARIATION, A NEW TOOL IN BREEDING
\L.E. Heszky, Li Su Nam, I. Simon-Kiss, K. Lökös, G. Gyulai, E. Kiss\
Acta Biologica Hungarica, 40 (4): 381-394, 1989.

The organ-specific somaclonal variation means the differences between the variability of somaclones originated from different somatic tissues of plant. Significant differences in some agronomical characters were achieved among somaclones of seed and plumule meristem origin.
The ploidy-dependent somaclonal variation means the differences between the variability of somaclones originated from different ploidy-level tissue. Increased variation among regenerated plants was postulated by origin from cultured cells of reduced ploidy level. The comparison of somaclonal variation in the progenies of diploid plants regenerated from callus of haploid and

diploid origin apported the ploidy dependent theory.
The pollenhaploid somaclone method (PHS-method) was developed and tested for utilization somaclonal variation in rice breeding. The PHS-method comprises the two well-known and widely applied in vitro methods which are the androgenesis (anther culture) and genetic instability of cultured haploid somatic cells (callus cultures). Developmental varieties produced by this breeding scheme are under certification in Hungary.

POLLEN BIOTECHNOLOGY AND ITS UTILIZATION FOR CROP IMPROVEMENT
\B. Barnabás, G. Kovács\
Characterization of Male Transmission Units in Higher Plants, Martonvásár, 37-40, 1990.

The brief consideration of some aspects of pollen biology indicated that the progress made so far in the field of structural and functional aspects of pollen is just the beginning. The new discoveries have brought about the realization that pollen has potential application in practical biotechnology. The method for wheat anther culture has already been incorporated into the wheat breeding strategies in Agricultural Research Institute.
Limited progress has been made in the laboratory of the institute in maize anther culture and in elaboration of more effective and less harmful rediploidizing methods for wheat haploids.

SPERM ISOLATION FROM WHEAT (TRITICUM AESTIVUM L.) POLLEN
\B. Barnabás, K. Liszt\
Characterization of Male Transmission Units in Higher Plants, Martonvásár, 37-40, 1990.

The male gametophyte has a great biotechnological importance. Artificial fertilization by isolated female and male gametophyte could solve the problem of incompatibility following the pollination. Sperm cell protoplasts (gametoplasts) might be very useful for genetic manipulation and fusion studies. Viable sperm cells have been isolated so far from Plumbago, Brassica species. In the scientific literature there are only few reports on attempts in isolating viable sperm cells from wheat. In our experiment two kinds of osmotics (sucrose and sorbitol) were used for wheat sperm cell isolation. The results show that both viable (FCR positive) but not normally shaped (spherical), and FCR negative but normal (spindle draped) sperm cells are available with the changing of quality and concentration of the osmotics. The isolated sperm cells kept their viability for 15 min. after isolation. As these short lived gametes are not suitable for in vitro fertilization experiments, further efforts are made to increase the efficiency of our method. However, the spindle- shaped sperm cells are applicable for ultrastructural studies.

POLLEN MATURATION IN FLORET CULTURES OF WHEAT (TRITICUM AESTIVUM L.)
\B. Barnabás, K.Liszt\
Characterization of Male Transmission Units in Higher Plants, Martonvásár, 59-61, 1990.

Floret cultures of wheat were established to study the effects of the in vitro conditions on pollen development. The incubated florets completed flowering within 7-9 days after inoculation. The in vitro environment influenced the normal pollen development and the number of immature and abnormal forms was increased. Using in vitro matured pollen for artificial pollinations the seed set was drastically reduced. Our experimental system seems to give opportunity to analyse pollen development processes under artificial conditions and to control and manipulate the double fertilization.

CYTOLOGY OF WHEAT (TRITICUM AESTIVUM L.) POLLEN DEVELOPED IN VITRO
\B. Barnabás, K. Liszt\
Characterization of Male Transmission Units in Higher Plants, Martonvásár, 63-68, 1990.

Cytological, histochemical and ultrastructural observation were taken to compare the structure and function of the pollen matured in vivo and in vitro. Analysing the semi-thin sections, the sperm cells and the vegetative nucleus showed normal morphological appearence in both cases, which indicates that the majority of these pollen grains could complete fertilization. On the basis of the histochemical examination some differences were observed in vivo and in vitro. The in vitro circumstances seemed to act as an environmental stress, consequently the lipid content was increased in the pollen cytoplasm indicating the stress response. Related to pollen wall formation there was an obvious difference: the intine layer of the in vitro matured pollen grains was thickened.

SOMACLONAL VARIATION IN DURUM WHEAT (TRITICUM DURUM DESF.)
\F. Sági, B. Beke, L. Sági\
Biotechnology in Agriculture and Forestry, 13: 494-510, 1990.

GENOTYPE DEPENDENT RESPONSES OF WHEAT VARIETIES TO WATER AND SALT STRESSES IN VITRO
\G. Galiba, L. Erdei, L. Sarkadi, A. Salgó, G. Kocsy\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, June 24-29, 1990.

Callus cultures of six varieties of wheat (Triticum aestivum L.) differing in drought and salt tolerance were maintained on media containing various concentrations on mannitol and NaCl as osmotic and saline conditions, respectively. To reveal genotype dependent adaptive responses, growth, total N, total P, Na^+ and K^+ concentrations, free amino acids, polyamines, protein content and exopeptidase activity were determined. The following factors proved to be important for the selection of osmotic and salt stress tolerant varieti-

es growth, total N and total P, Na^+ accumulation or exclusion, K^+ accumulation or loss, free amino acids and polyamines accumulation, decreased protein content and increased exopeptidase activity. Results suggest that callus cultures give genotype dependent responses under osmotic and salt stress conditions, and in general, they follow the adaptive responses characteristic at plant level.

DEVELOPMENT OF NEW RICE VARIETIES BY POLLEN HAPLOID SOMACLONE METHOD: RESULTS OF FIELD TEST
\L. E. Heszky, I. Simon-Kiss, K. Lökös-Tóth, G. Gyulai, E. Kiss, I. Geczky\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, June 24-29, 1990.

Several thousands of somaclones were produced from cv. Nucleoryza and Karolyna by PHS method in 1983-84. The new varieties of somaclone origin (HSC-1, HSC-2) were developed by classical breeeding method during the last four years. HSC-1 and HSC-2 showed promising results in field tests, HSC-2 even gave significantly higher yield than the standard and other varieties participating in the certification experiments in Hungary.
Pollenhaploid somaclone (PHS) method consists of the following steps: a\ reduction of ploidy level (androgenesis, gynogenesis) b\ maintenance and propagation of somatic tissue at reduced ploidy level, c\ production of somaclones from somatic tissue of reduced ploidy level. Callus is induced from the somatic tissue of haploid plants and after several subcultures diploid plants (pollenhaploid somaclones) are regenerated.

A NOVEL HORMONE-SELECTIVE AUXIN AND CYTOKININ BIOASSAY
\G. Gyulai, L.E. Heszky, K.T. Lökös, Zs. Jekkel,\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, June 24-29, 1990.

An in vitro auxin and cytokinin hormone selective bioassay has been developed. The system is based on an auxin-dependent adventitious root-, and a cytokinin dependent adventitious shoot induction of tobacco leaf discs, under sterile conditons, on agar medium, by the dark incubation time of 21th day. Auxin and cytokinin type (PGRs) reaction have been applied at a concentration range of 1 nanomolar - 1 mmolar. Two type of hormone reactions have been scored: 1\ a quantitative, aspecific callus-, and 2\ a qualitative, hormone selective morphogenesis induction.
The test system, in the case of exogenously applied cytokinin to be tested, takes the endogenous auxins in leaf discs as a given content and vica versa in the case of auxins applied. Consideration of this complementer hormone interaction enables screen selectively the new PGRs for auxin and cytokinin type reactions.
It has been postulated if a compound under test exerts auxin\cytokinin type effect it has to have a morphogenesis inductive concentration. In line with the bioassays from the very beginnings (Darwin 1896) to the recent days the

presented bioassay has been the first one which separates auxin and cytokinin type reactions selectively,precluding synergic and cross reactions. Patented, Hungary No. 4438\88.

TRANSFER TEMPERATURE CRYOPROTECTANT AND HOLDING TIME EFFECT IN THE SURVIVAL RESPONSE OF CRYOPRESERVED CELLS (PUCCINELLIA DISIANS L. PARL)
\Zs. Jekkel, L.E. Heszky, A.H. Ali\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, June 24-29, 1990.

Reflexed saltmarsh-grass suspensions have been frozen to subzero temperature before plunging into liquid nitrogen in a 1 °C\min freezing regime. The effect of cryoprotectants and holding time was studied at different transfer temperatures. Proline was the most effective protectant among the tested chemicals, generally in higher concentration. The optimum concentration of cryoprotectant varied at different transfer temperatures, showing that under different osmotical circumstances the occurence of protective dehydration depends on the current transfer temperature level. The lower cryoprotection of dymethyl sulphoxide and glycerol were raised by holding the cells for some minutes at transfer temperature before immersion in the liquid nitrogen. The modification of the adequate holding time followed the transfer temperature changes. Using holding time the frequency of callus capable for regeneration was improved.

NEOMORPH AND LEAF DIFFERENTIATION AS ABORTIVE PATHWAYS OF MORPHOGENESIS IN SOYBEAN (GLYCINE MAX L. MERR.) TISSUE CULTURE
\E. Kiss, L.E. Heszky, G. Gyulai, Zs. Horváth, A. Csillag\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, 24-29 June, 1990.

Somatic embryogenesis and organogenesis was induced on immature soybean cotyledons with using 2,4-dichlorophenoxy acetic acid and naphtalene acetic acid. On the basis of literal result and our experiments the conclusion can be drawn that the maintenance of normal processes of plant regeneration involves much greater difficulties that the induction of morphogenesis after dedifferentiation. Because of disturbances in ontogenesis in most cases plant can not be regenerated from the differentiated embryo-like structures and shoot meristems. Light and electronmicroscopic study of the embryo-like structures similarity showed that despite their morphological similarity to a zygotic embryo they should be regarded rather as neomorphs than embryos. They don't posses the two meristem centres characteristic for the embryo. The adventitous organs developing in the cultures are such shoot tips, only whose side meristem is active, so leaf primordia start developing from them.

HIGH EFFICIENCY ADVENTIVE EMBRYOGENESIS ON ANDROGENIC - AND SOMATIC EMBRYOS IN CHESTNUT (AESCULUS HIPPOCASTANUM L.) TISSUE CULTURE
\J. Kiss, L.E. Heszky, E. Kiss, G. Gyula\
7th International Congress on Plant Tissue and Cell Culture, Amsterdam, June 24-29, 1990.

Adventive embryogenesis - without callus phase - was succesfully induced from somatic cells of zygotic embryos on MS medium supplemented with BA and NAA.
Efficiency of cloning attainable by means of direct adventive embryogenesis was as high as 20 x during one month. The procedure has been proved succesfully applicable for the in vitro multiplication of pollen embryos and somatic embryos regenerated with low frequency from anther and callus cultures. Succesful adventive embryogenesis and its rate doesn't depend on the ploidy level and the origin of the primary embryos (zygotic, pollen and somatic), but it does depend on the developmental stage. Primary embryos are capable of embryogenesis, though the rate is different. The higher BA concentration (5-10 mg\l) increased the rate of adventive embryogenesis and accelerated their development. The highest proliferation rate of adventive embryogenesis (26 x) was achieved at hormon concentration of 10 mg\l BA and 1 mg\l NAA. The steps of the development of the adventive embryos were followed by scanning electron microscope. The method has the particular adventage, that the embryos are capable of secondary and tertiary embryogenesis, additionally the adventive embryos can also be propagated by means of this method. This means, that practically unlimited great number of adventive embryos - genetically identical with the primary embryos - can be obtained independently on ploidy level from embryo cultures available in limited number within a short time.

RESTORATION OF THE REGENERATION POTENTIAL OF LONG-TERM CELL CULTURE IN RICE (ORYZA SATIVA L.) BY SALT PRETREATMENT
\Do Quang Binh, L.E. Heszky\
Journal of Plant Physiology, 136: 336-340, 1990.

Cell suspension, initiated with seed-derived embryogenic callus and maintained for 2 years, produced morphogenically different cell types. No plant was regenerated from fine and friable cells. Low and sporadic regeneration frequency (0,0-0,3%) was observed in compact cell clusters. Friable cells (20-21 months old) were exposed to salt stress, 0,75 % NaCl mainly inhibited cell growth and induced cells to become compact, whereas 1,5 % NaCl expressed a selective effect of killing all but embryogenic cells of friable cultures. A 3 month pretreatment with 1,5% NaCl resulted in a reselection of embryogenic cells, restoring the high regeneration capacity of 2-year old cultures of Oryzella by 60% and Karolina by 37% on salt-free medium. NaCl used at a suitable concentration appeared to be an efficient factor for reestablishment of the regeneration capacity of embryo-

genic calli derived from cell culture in rice.

BIOCHEMICAL CHANGES INDUCED BY OSMOTIC STRESS IN WHEAT CALLI
\G. Galiba, L. Simon-Sarkadi, A. Salgó, G. Kocsy\
20th Meeting of the Federation of European Biochemical Societies, Budapest, August 19-24, 1990.

Callus cultures of four varieties of hexaploid wheat (Triticum aestivum L.) were maintained on media containing various concentrations of mannitol. The induced osmotic stress inhibited growth and increased the percent dry matter and the level of free amino acids of the calli. Bigger changes were observed in drought sensitive and moderate resistant varieties than in drought resistant ones. The putrescine content was highly increased in the drought sensitive variety. The extractable protein content was decreased in drought sensitive and in moderate resistant varieties. During osmotic stress the aminopeptidase and carboxipeptidase activity increased significantly in the drought sensitive variety.

ADAPTATION OF WHEAT TISSUE CULTURE TO WATER DEFICIT AND COLD STRESS
\G. Galiba, L. Simon-Sarkadi, A. Salgó, L. Erdei, G. Kocsy, J. Sutka\
Physiologia Plantearum, 79: A111, 1990.

Callus cultures of wheat varieties (Triticum aestivum L.) differing in frost and drought resistance, and the calli of chromosome substitution, and alloplasmic lines were cultured on media containig mannitol and polyethylene glycol as osmotic agents. Growth, minerals, free amino acids, polyamines, protein content, exopeptidase activity and the activity of some of the direct oxidases were determined to reveal genotype dependent adaptive responses. The effect of abscisic acid on frost resistance was also investigated and compared to the degree of frost resistance developed after cold hardening.

POLLEN SELECTION: THEORETICAL BASES AND BREEDING PROSPECT OF THE METHOD
\G. Kovács, B. Barnabás\
Növénytermelés, 38:347-354, 1989.

A brief survey is offered of a trend of pollen biological research that in our days is showing intensive development. The biological bases of pollen selection, the present situation - successes and problems - as well as prospects of relating researches are summed up.

SELECTION FOR COLD HARDINESS IN POLLEN POPULATIONS OF MAIZE
\G. Kovács\
Növénytermelés, 38: 9-13, 1989.

Several authors have reported a positive correlation between the performance of the pollen and of the parent sporophyte. Pollen grains of genotypes tolerant to unfavorable conditions such as low\high temperature, salinity, agrochemicals and pathotoxins also show such tolerance, they can germinate even unfavorable conditions. The large

population sizes in combination with the haploid genetic systems constitute a great potencial in carrying out selection on pollen level. The direct exposure of pollen to environmental stresses can cause changes in the frequencies due to selection pressure, consequently pollen selection can be used in crop improvement. Cold storage of maize pollen at 4 °C seemed to be a satisfactory treatment to select pollen population to cold tolerance. The seeds set by cold treated pollen germinated in a greater number under low temperature condition than the control. During the cold storage probably the more tolerant pollen grains survived and transmitted this tolerance to the next generation. In conclusion we suggest that pollen selection may present a new important breeding tool and could be easily incorporated into many breeding programs.

EFFECT OF COOLING RATE, CRYOPROTECTANT AND HOLDING TIME AT DIFFERENT TRANSFER TEMPERATURES IN THE SURVIVAL OF CRYOPRESERVED CELL SUSPENSION CULTURE (PULCINELLIA DISTANS \L.\ PARL.)
\L.E. Heszky, Zs. Jekkel, A. Abdel-Hamid\
Plant Cell, Tissue and Organ Culture, 21: 217-226, 1990.

Reflexed saltmarsh-grass suspension cultures produced by seed callus were frozen to the liquid nitrogen temperature. Cooling rates, cryoprotectants and holding times were taken as a function of transfer temperatures. The highest survival of cells (45%) was found at a freezing rate of 1 $^{0}C\ min^{-1}$, without cryoprotectant treatments. The cryoprotectants (proline, dimethyl sulphoxide, glycerol) used at different concentrations and transfer temperatures, increased the survival rate. The maximum value was 78% at 12,5% (w\v) of proline with -30 °C transfer temperature. Considerable improvement of viability (from 0% to 95%) among the 12,5 and 15,0% (v\v) dimethyl sulphoxide cryopreserved cells was achieved by holding them at -20 °C for 10-30 min before plunging into the liquid nitrogen. A 20 min holding time at 15,0% (v\v) glycerol level and -30 °C transfer temperature significantly enhanced the viability of the explants from 42% to 92%. Plants were successfully regenerated from cells cryopreserved with proline (w\v) and dimethyl sulphoxide (v\v) levels of 12,5 and 15,0%, respectively.

A NEW, ENDOSPERM-SUPPORTED CALLUS INDUCTION METHOD FOR WHEAT (TRITICUM AESTIVUM L.)
\T. Bartók, F. Sági\
Plant Cell, Tissue and Organ Culture, 22: 37-41, 1990.

A new, endosperm-supported callus induction method was developed using mesocotyls of mature wheat embryos. After seed germination under aseptic condition, most of the germ tissues were cut off and only a few mm of the mesocotyl tissue with the scutellum was used for callus induction. The seeds were placed furrow downwards in 2,4-D solution (6-8 mg\l). Proliferating callus tissues were already observed on the cut surface of the mesocotyls on the 2nd day after inoculation. On the MS nut-

rient medium, callus formation from the isolated scutella with attached mesocotyls was negligible even after 6 days. For shoot and root regeneration, the calli produced up to 10 days were removed from the seeds and transferred onto a hormone-free MS medium. As shown by histological methods, the plantlets regenerated via organogenesis.

DISEASE SYMPTOMS IN TRANSGENIC TOBACCO INDUCED BY INTEGRATED GENE VI OF CAULIFLOWER MOSAIC VIRUS
\E. Balázs\
Virus Genes, 3 (3): 205-211, 1990.

A chimeric vector (pKR 612B1) containing the neomycin phosphotransferase (APH) gene from the Tn5 transposon under the control of the gene VI promoter of cauliflower mosaic virus (CaMV) and the cloned gene VI region (SalI-BstEII) of the same virus were used to cotransform tobacco protoplasts. Using the polyethylene glycol transformation procedure, a large number of protoplasts were transformed and proved to be resistant to kanamycin (Km). Whole Km-resistant plants were regenerated and shown to contain the integrated foreign genes. DNA from transformed clones was analyzed by Southern blot hybridization, showing the presence of the Tn5-derived gene and the viral gene. Transgenic plants containing the viral gene show mild mosaic patterns and fasciation. The expression of the gene VI product was detected by immunoblots.

AN IMPROVED SYSTEM TO OBTAIN FERTILE REGENERANTS VIA MAIZE PROTOPLASTS ISOLATED FROM A HIGHLY EMBRYOGENIC SUSPENSION CULTURE
\S. Mórocz, G. Donn, J. Németh, D. Dudits\
Theoretical and Applied Genetics, 80: 721-726, 1990.

Regenerants from a 30 month-old haploid and a 10 month-old diploid tissue culture were cross-pollinated to generate a synthetic genotype (HE\89) with improved competence for maintenance of totipotency in various cultured explants. The HE\89 zygotic embryos developed friable, embryogenic cultures in the commonly used MS- and N6-based media without the addition of L-proline. By optimalization and changing the culture conditions, we were able to regulate the maintenance of the earlier, more synchronous (Type II) and the later, asynchronous (Type I) in vitro embryogenesis, as well as the shift between different ontogenic stages. Within 70 days after the inoculation of immature embryos a relatively homogenous, early embryogenic suspension culture usable for protoplast isolation was established from the initially surface-grown cultures. Using modified solutions for protoplast isolation and culture, viable protoplasts were reproducibly obtained from which plants were regenerated via defined ontogenic steps. Despite the long in vitro history of the parental genotypes, 60-70% of the more than 500 plants derived from the HE\89 protoplasts set seeds following self- or sib-pollination.

ANIMAL BREEDING

ROLE OF BIOTECHNOLOGY IN THE SPHERE OF VETERINARY VACCINE PRODUCTS
\F. Sólyom\
Acta Biotechnologica, 10 (6): 479-483, 1990.

During the last century two kinds of methods were developed in the field of vaccine production: those of inactivated and of attenuated vaccines. Both of them contained the whole microorganism. Thus, postvaccinal lesion even sporadically might occur. In the last 15 years, due to the development of molecular biology and of genetic engineering, novel technologies of vaccine production dissimilar to the former ones were and are developed, which are as follows: subunit, synthetic, recombinant, genetically engineered and antiidiotype vaccines. Apart from genetically engineered vaccine, vaccines produced by means of new methods contain merely the immunocompetent protein particle of microorganism, thus, postvaccinal lesion should not be expected using vaccines of these kind. Vaccines produced by means of primary biotechnologic methods were put on the market in 1983 and introduction of 25 to 30 vaccines is expected until 1992. Approximately 145 companies, institutes are dealing worldwide with development of veterinary vaccines and they are in competition, with enormous investment capital, for accession of share as much as possible in vaccine market of about 550 million dollars.

ROLE AND FUTURE DEVELOPMENT OF INTERNATIONAL INTEGRATION IN ANIMAL BREEDING
\J. Dohy\
Állattenyésztés és Takarmányozás, 38 (6): 481-483, 1989.

SEVERAL ASPECTS OF WIDER INTERPRETATION OF GENE EROSION IN ANIMAL PRODUCTION
\J. Bögre, J. Dohy\
Állattenyésztés és Takarmányozás, 39 (2): 99-101, 1990.

BIOTECHNOLOGICAL RESEARCH IN THE RESEARCH INSTITUTE FOR ANIMAL PRODUCTION
\T. Gere, S. Holdas, L. Wekerle, I. Szalay, M. Papp, I. Veres\

Állattenyésztés és Takarmányozás, 39 (5): 385, 1990.

The authors survey the biotechnological research conducted in the Research Institute for Animal Production. Report is given on the position of embryo transfer and on outlooks of its application in the Aujeszky's disease eradication programmes. Their method for in vitro fertilization is also disclosed. Examinations on the effect of vitamin A end beta-carothene on reproduction are summarised too.
Production of sex-oriented semen was attempted by using the difference in capacity for binding lectine and by separation of the sperms in cervical mucus. Examinations conducted in connection with the spontaneous parthenogenesis of geese are also reported.
Finally, the authors summ up the results obtained so far in respect of separation the bovine growth hormone gene and examination of its RFPL (restriction fragment lenght polymorphism).

POSSIBILITIES OF BIOTECHNOLOGY APPLICATION IN THE HUNGARIAN JOGBREEDING
\B. Bényei, Gy. Krix\
Biotechnical and Biotechnological Methods in the Intensification of the Animal Production, Kosice, September 18-20, 1990.

At present 250 000 dogs live in Hungary. They are the object of the small-scale breeding for the most part. The principal task of the reserach are as follows: adaptation of the method and its application in the cryoconservation of sperms, induction, synchronization and detection of the rut, embryo-transfer and insemination in vitro. The ultrasonic and laparoscope examinations are used to determinate exactly the ovulation. It is necessary to inject GnRH every 90 minutes in order to insure the fertilization rate which is realized by the automatic syringe fixed on the animal back. The results of embryo-transfer are modest up to date in comparison with those of foreign institutes. The aim of the research is the qualitative improvement of the dog-breeding and use of new methods also for other carnivores.

APPLICATION OF BIOTECHNOLOGY IN THE HUNGARIAN HORSE-BREEDING
\F. Szász, J. Juhász\
Biotechnical and Biotechnological Methods in the Intensification of the Animal Production, Kosice, September 18-20, 1990.

Hungary is known by rich traditions in the horse-breeding. At present still more horses are bred for sport and other interest aims. The artificial insemination began in Hungary 10 years ago, but unfortunately without any practical importance. The methods has found its real application only nowadays. 7 insemination stations work here where 350-400 mares are inseminated with fresh dilute sperms a year. In these centres the vaginal and rectal examination, determination of progesterone and the ultrasonic apparatus are used for diagnostics of oestrus cycles. It is investigated the problems of cryoconservation of sperms having 7 000 frozen insemination doses from 7 breeds. The emb-

ryo-transfer began working here 2 years ago, too, though meantime without any significant results. Except of the main research the centre of Üllő organizes the courses for insemination technicians, students and veterinarians as well.

BIOTECHNOLOGICAL RESEARCH OF THE ANIMAL PRODUCTION OF BUDAPEST UNIVERSITY OF VETERINARY SCIENCES
\J. Seregi, F. Szász\
Biotechnocal and Biotechnological Methods in the Intensification of the Animal Production, Kosice, September 18-20, 1990.

The research of the University of Veterinary Sciences concentrates on the improvement of the animal production, in the that of cattle, horses, pigs and sheep, as on the practical use of transfer and bisection of embryos and modern biotechnological methods. The embryo transfer has reached the number of 3000 in cattle and 2500 in sheep. The fertilization in vitro succeeded in the birth of 5 calves and other newborn animals are expected. As concerns the horses the reproduction is prepared for pig and goat breedings. The important aim of the reserach is to preserve the Hungarian gene potential for all breeds of animals.

NEW POSSIBILITIES AND PROSPECTS FOR THE GLOBAL BREEDING STRATEGY
\J. Dohy\
International Symposium, Teheran, 1990.

EMBRYO TRANSFER AS A POSSIBILITY FOR THE ERADICATION OF AUJESZKY'S DISEASE IN SWINE
\J. Haraszti, I. Medveczky, G. Rónay, J. Seregi, L. Solti, J. Varga\
Magyar Állatorvosok Lapja, 44 (6): 325-327, 1989.

The role of Aujeszky's disease virus infected embryos was investigated in the transmission of the infection. During the first experiment, donor sows were infected by a dose of 3×10^7 $TCID_{50}$ of Aujeszky's disease virus by intranasal and intravaginal routes at the time of insemination. During the second series of experiments, 6 gilts were infected at the beginning and parallel with the hormone treatments. The third series of experiments were carried out under field conditions on a state farm.
The experiences obtained during the experiments have shown that the transmission of embryos, originating from experimentally infected donors, can be transferred without any risk of the transmission of infection even then when recovery of zygotes was carried out from the donors during the state of acute viraemia. 0,25 % trypsin treatment of zygotes recovered from infected uterinal environment and a subsequent washing procedure in Dulbecco's solution prevented the transmission of infection. This was also confirmed by the lack of seroconversion in recipient sows tested within 5o days after embryo transfer, as well as in newborn pigs. In the course of the field experiment, all the five recipient sows also remained seronegative during the repeated serological examinations carried

out within 50 days after embryo transfer.

TASKS AND POSSIBILITIES IN THE SAVING OF GENERESERVES IN THE ANIMAL BREEDING BY MODERN METHODS
\I. Bodó, Gy. Kovács, J. Seregi, E. Takács\
Magyar Állatorvosok Lapja, 9: 517, 1990.

THE USE OF THE SUPEROVULATION, EMBRYO TRANSFER AND NUCLEUS CULTIVATION IN THE CATTLE BREEDING
\G. Brem, J. Seregi\
Magyar Állatorvosok Lapja, 11: 653, 1990.

GENE EROSION - GENE CONSERVATION
\J. Dohy, J. Bögre\
Magyar Mezőgazdaság, 45 (6): 16-17, 1990.

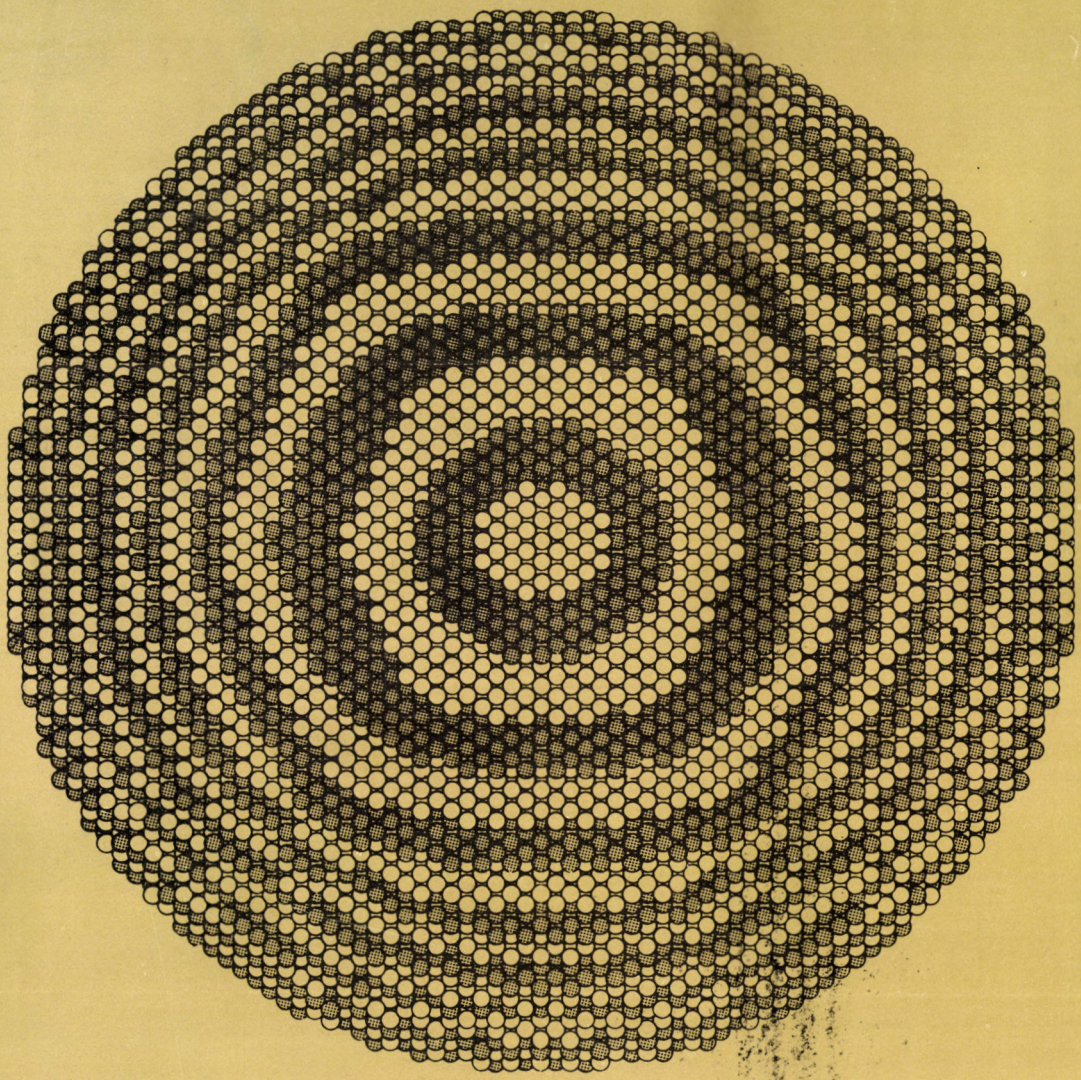
A MODEL EXPERIMENT TO STUDY THE TRANSMISSION OF AUJESZKY'S DISEASE VIRUS (ADV) IN SWINE EMBRYO TRANSFER
\I. Medveczky, L. Solti, J. Haraszti, G. Rónai, K. Ekés, S. Belák, E. Tury, J. Varga, J. Sántha, J. Seregi\
Proceedings of the 11th IPVS Congress, Lausanne, July 1-5, 1990.

Four sero-negative sows from a farm free of AD were infected both intranasally and intrautero with a virulent ADV strain six days before excision of embryos.
The virus was reisolated from nasal and vaginal swabs and from different part of the genital organs (ovary, oviduct, cervix, uterus) of all infected sows. The solution used for washing the oviduct also contained infectious virus after the excision of embryos in case of two infected sows. The results of virus isolation was confirmed by dot-blot hybridisation and histological survey as well.
All embryos were treated with trypsin solution according to the proposed method of I. E. T. S. before implantation. The embryos from 4 sows with intact zona pellucida were implanted into one AD free recipient sow.
The recipient sow and the offspring remained sero-negative indicating that the transmission of ADV was blocked during the embryo transfer.

BACTERIAL CONTAMINATION OF THE UTERUS AFTER PARTURITION AND ITS EFFECT ON THE REPRODUCTIVE PERFORMANCE OF COWS ON LARGE-SCALE DAIRY FARMS
\T. Takács, I. Gáthy, Z. Macháty, E. Bajmócy\
Theriogenology, 33 (4): 851, 1990.

The bacteriological status of the uteri of 150 cows was examined on 14 large-scale dairy farms 10 to 20 d after parturition and then twice again in 2-wk intervals during uterine involution. The degree of bacterial contamination and the proportion of the infected uterus as well as the composition of the isolated flora were determined. The antibiotic sensitivity of the important bacterium strains was also studied. The relationship between the reproductive data and bacterial contamination of the uteri was analyzed, and the influence of certain

environmental and genetic factors on the rate of infection was examined.
The proportion of cows having moderate or more serious uterine infection remained above 30% at the end of involution on more than 35% of the farms. Infections were caused mainly by streptococci, E. coli and corynebacteria. The bacterium strains showed broad resistance against commercially available antibiotics. There were significant differences in the length of time from the parturition to the first insemination and conception among cows at the various farms. There was a significant correlation between these differences and the bacteriological status of the uteri. Reproductive data were the lowest in the group of cows infected with Corynebacterium pyogenes.
The rate of the infected uteri was considerably higher after the third parturition and in cows producing less than 20 l of milk per day. No connection was found between the bacteriological status of the uteri and the breed, type of housing, and season of the parturitions.



ORSZÁGOS MŰSZAKI FEJLESZTÉSI BIZOTTSÁG
FEHÉRJE- ÉS BIOTECHNOLÓGIAI IRODA